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MALARIA CONTROL ACTIVITIES OF THE WORLD HEALTH ORGANIZATION[1]

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Internationalism is an arresting feature of modern public health practice. Amnog its many compelling aspects is that which embraces the activities of the World Health Organization in the field of malaria control, and this is the topic of the present paper.

To a measurable degree, the origin of the WHO malaria program may be said to lie in the famous Malaria Commission of the old League of Nations Health Organization. The latter had been set up to make it easier for certain advanced countries to share newer and more effective disease prevention techniques with underdeveloped areas. Such matters as quarantine reform, standard vital statistics, medical research, and control of epidemics received much attention. Many authorities believed that the League of Nations was more successful in the sphere of international public health than in other aspects of its program.

That the World Health Organization is the spiritual heir of the League of Nations health agency may be presumed on the basis of declarations in the new WHO Constitution. Here it is written that the objective "shall be the attainment by all peoples of the highest possible level of health." Certain functions are prescribed to enable the organization to reach this goal. For example, scheduled activities include assisting governments upon request to strengthen health services by furnishing technical assistance, providing consultants, specialists, travel grants and fellowships. Other aims are standardizing diagnostic procedures, nomenclatures and quarantine; collecting and disseminating health statistics; fostering research; aiding the development of more effective administrative, diagnostic and prophylactic techniques; promoting improved standards of teaching and training in public health and related professions; and acting as the directing and co-ordinating authority on international quarantine and health measures.

Before discussing details of malaria control phases of the WHO program, it will be useful to outline briefly the structure of the organization itself. First it may be noted that WHO is one of several specialized agencies affiliated with the United Nations. It was first visualized in San Francisco in 1945 at the United Nations Conference, where the basic importance of public health was recognized and this subject was listed among those of major interest. A Technical Preparatory Committee of experts met in Paris early in 1946 and prepared a Draft Constitution. Later in the same year an International Health Conference convened in New York, at which 51 nations co-operated in setting up a World Health Organization Interim Commission. By April 7, 1948, the WHO Constitution had been ratified by 26 member states of

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the United Nations and thus became activated. WHO came into being officially on the following September 1 when the Interim Commission was formally transformed into the full-fledged World Health Organization, now having 67 members.

The work of WHO is carried out by three bodies; namely, (1) the World Health Assembly, consisting of delegates from each member nation, (2) the Executive Board, and (3) the Secretariat. The first Assembly met in Geneva in 1948 and the second in Rome in 1949. These assemblies determine organization policies and take appropriate action to accelerate progress towards the objective. The Executive Board of 18 members functions to give effect to decisions and policies of the Assembly. The Secretariat, headed by a Director General, consists of technical and administrative personnel. There are two Assistant Director Generals, one for Technical Services and one for Operations.

Certain Expert Committees have been established by the Health Assembly. Members are appointed by the Director General, and they act as consultants in their respective fields.

Finally, it should be noted that within WHO there are Regional Organizations, each consisting of a Committee, an Office, and a Director. These local organizations formulate regional policies and supervise regional activities, thus decentralizing the field work of WHO. In the Americas, the Pan American Sanitary Bureau acts as the Regional Organization. Others are operating in the Eastern Mediterranean and in Southeast Asia, and another is planned for the Western Pacific area.

Turning now to more specific consideration of international malaria activities, it will be recalled that the old League of Nations Health Organization created an Advisory Malaria Commission which stimulated research in malaria epidemiology, the biology of plasmodia and the nature of immunity, and especially in malaria therapeutics. Malaria training schools were aided and new courses were set up in Singapore and in Europe. Many fellowships for junior officers and travel grants for senior officials were provided. Malaria was one of the principal topics for discussion at the two Intergovernmental Conferences convened by the League health agency, namely, the Pan African Health Conference in Johannesburg in 1935, and the Conference on Rural Hygiene in Bandoeng, Java, in 1937.

During the final years of the Second World War and in the immediate postwar period, when the League of Nations had disintegrated, the emergency agency known as UNRRA (United Nations Relief and Rehabilitation Administration) sponsored malaria control campaigns in Greece, Italy and the Far East, and it also supplied antimalarial drugs to many other countries. In 1947, the Interim Commission of the World Health Organization assumed the functions of the Health Division of UNRRA, including responsibility for completing certain malaria projects which UNRRA had carried forward so successfully. Thus, it appears that there has been at least a thin line of continuity from the Malaria Commission of the League Health Organization through UNRRA and the WHO Interim Commission to the present malaria program of the World Health Organization.

The long-range objective of WHO in respect to malaria is the world-wide eradication of this disease as a public health problem. The immediate objectives may be briefly summarized as (1) to prove to governments the economic feasibility of malaria

control, (2) to demonstrate the indirect benefits accruing in general public health and welfare and in increased agricultural and industrial production, and (3) to assist and encourage governments toward nation-wide control and eradication of malaria by well-organized, routine application of modern methods.

To achieve these aims, the present policy of WHO, within the limitations of its budget, includes certain practical health services to governments which request them. For example, experienced malariologists and/or demonstration malaria control teams are made available to give advice and practical assistance in setting up national antimalaria services and in training local personnel. This help is on a temporary basis but is usually planned for not less than two years in the case of demonstration teams. Governments are required to appoint local officers to understudy each member of such a team, which usually includes a malariologist, an entomologist and an engineer.

Another method of assisting governments is to aid schools of malariology and to establish WHO malaria courses in selected areas where such facilities are required. Also an important help is the provision of WHO fellowships and travel grants for training in malariology. Furthermore, WHO is preparing to collect, correlate and disseminate malaria data, to standardize techniques and measurements, and to collect and distribute aids to malaria control propaganda for public instruction. WHO is cooperating with UNICEF (United Nations International Children's Emergency Fund) in the Far East in attempts to reduce infant and child mortality and morbidity by malaria control. Finally, WHO is cooperating with FAO (Food and Agriculture Organization) in the selection of malarious areas of great food-producing potentiality, in order to demonstrate the agricultural benefits which may be derived from effective malaria control.

The WHO Interim Commission appointed an Expert Committee on Malaria and the latter held two meetings—the first in Geneva in 1947, and the second in Washington in 1948. After the termination of the Interim Commission and until a new WHO Expert Committee on Malaria was appointed, the Interim group served as an *ad hoc* committee, which held no meetings but was consulted by mail. The new WHO Expert Committee on Malaria was appointed this year and met in Geneva in August. It should be emphasized that WHO Expert Committees have no executive functions whatever: they act merely as advisory bodies on technical questions in their own fields.

Within the Operations Division of the Secretariat of WHO, there is a Malaria Section responsible for carrying out the malaria policies of the organization. When the Regional Offices are well established, they will provide direction for much of the malaria field work within their respective areas.

The budget of WHO is lamentably small, so that only about $375,000 is available for malaria projects in 1950. However, there is provision for a supplemental budget, so that if special funds are forthcoming from extra contributions by governments, some $750,000 may be provided for the malaria items.

During 1949, it has been possible for WHO to implement the following malaria control program in cooperation with 16 individual governments, and partly financed by UNICEF funds: (1) demonstration malaria control teams—one in Pakistan and

four in India; (2) survey teams—one in Siam and one in Afghanistan; (3) a full-time malaria consultant in Asia for the joint UNICEF-WHO projects; (4) short-term malaria consultants for Bulgaria, Yugoslavia, Hungary, Turkey, Greece, Israel, India, Iran, Ceylon, Mexico, Venezuela, and the United States; (5) equipment for the Malaria Institute of India for establishing new laboratory facilities for students; (6) 28 fellowships for basic or advanced malaria training; (7) a panel of some 46 corresponding malaria experts. Contact by correspondence will be maintained between the Secretariat and these experts in order to facilitate the collection and dissemination of malaria data.

Some conception of the international nature of WHO can be obtained from the fact that one malaria team in North Central India has been headed by a Greek malariologist. Another team, working in the Jeypore Hills in Central Eastern India, is led by a Canadian, while a third team, working in the Malnad area of Mysore, is in charge of an American.

Finally, mention should be made of the WHO Expert Committee on Insecticides, which held its first meeting in Sardinia in May, 1949. The report of this group, when published, will prove helpful in regard to specifications for sprayers, standards for insecticides, and methods of quarantine against mosquitoes.

The first and second World Health Assemblies have assigned first priority in the WHO program to malaria, along with tuberculosis, venereal diseases, infant and maternal hygiene, and environmental sanitation. The reason in the case of malaria is that by virtue of insecticides developed since 1940, it is now feasible anywhere in the world to attain a high degree of control. But the world-wide eradication of malaria can be achieved only when each and every government faced with the problem has put into effective operation an adequately financed antimalaria organization adapted to local conditions and needs, and properly staffed. In numerous places such programs are well advanced, and in special areas it is reasonable to predict the eradication of malaria as an endemic disease within the next few years. However, in a majority of malarious countries there is still great need for practical encouragement and help along lines of the WHO malaria control policy discussed above.

Surely, here is an international opportunity and venture which must arouse optimism, not only because of the basic objectives, but also because of potential collateral benefits in good will among nations. The Constitution of the World Health Organization states that, "The enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being, without distinction of race, religion, political belief, economic or social condition." It is hoped that adherence to such a principle may help nations to eradicate not only the plasmodia of malaria but also the even more dangerous seeds of international hate and political intolerance.

FINAL PHASES OF MALARIA ERADICATION IN THE UNITED STATES

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The five-year National Malaria Eradication Program of the State health departments and the U. S. Public Health Service, originally proposed by Dr. L. L. Williams, Jr. (Mountin 1944), was launched on July 1, 1947. Thus it has now reached its midpoint. Its goal is the elimination of malaria as an endemic disease from the continental United States. The tactical plan involves concurrent reduction of malaria parasites and vectors until the probability of malaria transmission becomes nil (Andrews and Gilbertson 1948). This is being effected by killing adult anophelines with residual insecticides in homes and by improved diagnosis and treatment of malaria. Residual DDT spray projects have been carried on jointly by State and Federal health agencies since the spring of 1945 and were continued in fiscal years 1948 and 1949. By the end of this period over 4,650,000 house spray applications had been made in the rural sections of as many as 347 counties per year (Operational Reports 1945–49). The selection of these counties was based on reported malaria mortality rates from 1938 to 1942, inclusive, suitable adjustments being made for later mortality experience. Spraying was also done in homes within a mile of houses in which verified cases of malaria occurred.

For the economic and effective direction of these insecticidal operations and for the detection and treatment of cases, reliable information concerning the distribution and prevalence of current malaria was needed. These attributes have been so low in recent years that spleen and blood surveys are no longer informative measures of their levels. With the exception of the postwar flurry of positive blood films from repatriated service personnel, State health laboratories have reported fewer and fewer confirmations of malaria morbidity each year. The only remaining sources of information were the case reports and death certificates of practicing physicians. In general these have been notably defective wherever checked in this country (Quinby 1950), though their trends undoubtedly have been related roughly to those of seasonal and geographic variations in the occurrence of paludism (Andrews 1941). Thus, efforts have been made and are still in progress to improve the quality of these reportorial data.

Accordingly, Public Health Service medical and nurse officers were made available in some of the southeastern States to amplify the State health department facilities in promoting more accurate diagnosis and reporting of cases, more effective treatment with modern antimalarial drugs, and more thorough investigations of deaths alleged to be due to malaria. Very real improvements have resulted though the situation is still far from perfect. Some of the States have shifted from accepting the vague estimates of practitioners concerning the malaria cases seen during the previous month to demanding the names and addresses of all persons treated for malaria; this has sharply reduced reported malaria morbidity. At least one State[1], historically

[1] Mississippi.

malarious, has offered a monetary bonus for each confirmed case of malaria—and was able to double the bounty the second year to stimulate further case-finding.

In the pursuance of these activities, it is probable that more effort was spent in trying to minimize the numbers of cases reported than in discovering new ones—though this last was by no means neglected. To compensate in some degree for such overemphasis, blood-film surveillance was—and still is—conducted almost continuously in the populations adjacent to cooperatively supported malaria observation stations (Bradley and Goodwin 1949). These are situated in areas representative of the three great physiographic-ecologic types of terrain associated historically with malaria in this country—the limestone area of the southeast, the environs of inadequately prepared impoundments, and the agricultural Mississippi Delta region. In each of these type locations, malaria has been severely endemic in the past. It is believed that any general resurgence of the disease would be manifested promptly in the vicinity of one or more of these stations. At present there is no evidence of new cases at or near any of these sampling outposts.

To improve laboratory diagnostic facilities for practitioners, refresher courses in the recognition and identification of malaria parasites have been offered since 1945 by the Communicable Disease Center in Atlanta to qualified personnel principally from State health and veterans' hospital laboratories (Anonymous 1949). Thus far, 270 technicians and 64 laboratory supervisors from all but two States have taken this training. The Center also maintains a reference diagnostic service to which difficult or questionable specimens may be sent for examination.

In spite of the introduction by returning troops of foreign but locally transmissible strains of plasmodia slpecies (Young, Eyles and Burgess 1948), the nation-wide decline in reported malaria case and death rates has continued steadily since 1936 as shown in Fig. 1. Unless an organized conspiracy of silence exists among practitioners, it would appear from these trends that we are closer to malaria eradication than we were two years ago—but the quality of these same data is not fine enough to indicate how far we are from the actual goal. On the basis of field evidence and experience, however, it seems reasonable to assume that we are in the final phases of the process. Thus it becomes desirable to consider program changes imposed by this circumstance or needed to achieve ultimate success.

In the first place it will be necessary to reduce the coverage of the residual spray activities. Reductions in Federal funds for this project were made during each of the last two years; another cut is imminent. Present Federal participation amounts to slightly less than half the total cost of the program. It seems unlikely, however, that State and local governments can be prevailed upon to contribute more in the future than they have in the past. Thus the number of States and counties in which the joint program of domestic spraying can be operated in the future will have to be curtailed. It is hoped that the termination of Federal aid in a given county may not necessarily mean the closing of the project; because with State assistance, some local governments should be able to continue residual spray operations. Where Federal assistance for general field insecticidal activities is withdrawn from a State, it is planned to maintain at State level a one- or two-man team to provide surveillance for a period of years. The activities of these teams would be (a) to encourage and supervise spot-

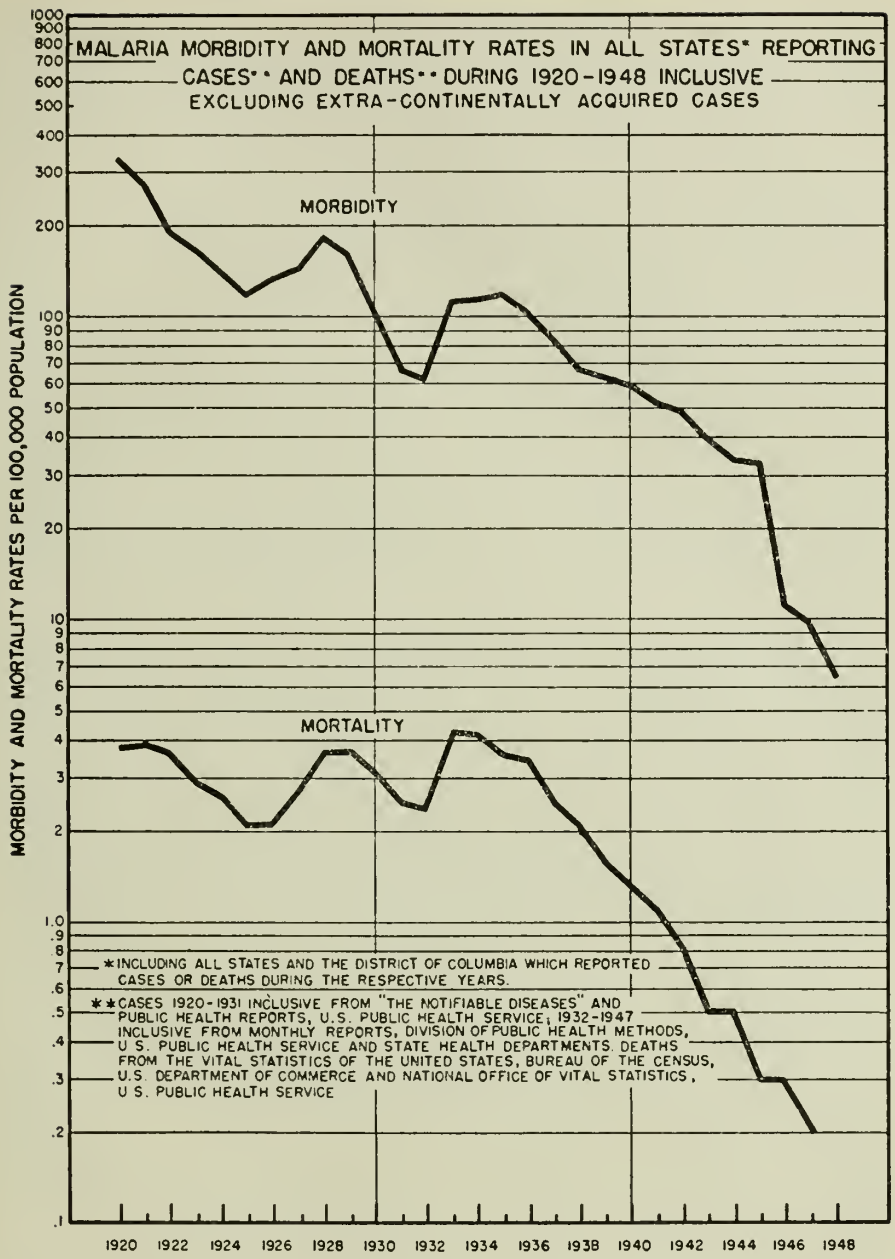

FIG. 1

spraying around reported malaria cases, (b) to furnish technical consultation to local governments on drainage, insecticidal and similar activities, and (c) to keep alert for evidence of returning of malaria.

The reduction in the number of approved counties does mean that the Federal-

State spray program must become more selective. In the past, it has blanketed rural sections of counties in which reported malaria death rates have been above specified levels. This must be continued in the future where possible; but the highest priority should be assigned to "mopping-up" operations, i.e., the residual spraying of homes in areas surrounding *bona fide* cases of malaria.

Thus it becomes more imperative than ever that malaria morbidity and mortality data should be secured and reported with the greatest possible accuracy. It seems anomalous in this enlightened age of specific and supporting antimalarial therapies and of widespread medical service that anyone should die from malaria in this country; yet 214 deaths—the lowest number reported this century—were certified as due to malaria in 1947. Deaths should not be ascribed to malaria on the basis of hasty clinical impression or because no other cause seems obvious; they should be attributed to malaria only if the attending physician has established beyond reasonable doubt that malaria was the actual cause of death. More circumspection by practitioners in this regard should be urged in medical societies and journals. This accomplishment plus careful investigation of alleged malaria deaths by State health officials, and correction of death certificates where indicated, should hasten the day when "malaria deaths" disappear from the public health records of this country.

If and when that day arrives, it is evident that mortality data—hitherto regarded as the most valuable of our malaria statistics—can be used no longer as an indicator of malariousness. Total reliance will have to be placed on morbidity, and its reporting must be improved accordingly in accuracy and adequacy. To achieve this, three common misconceptions among practitioners should be corrected: one, that most cases of malaria can be recognized unerringly by clinical evidence alone; two, that blood films—thick or thin—rarely reveal the presence of parasites; and three, that all fevers which subside after the administration of antimalarial durgs are malaria.

Diagnosticians should try to distinguish clinically between malarial illness and the array of other disorders which, especially in their prodromal stages, resemble malaria —but clinical judgment should be verified by laboratory test whenever possible. Thick-film diagnosis is admittedly imperfect but it is the most dependable method thus far available. It must be more reliable than is generally conceded judging by the comparative ease and regularity with which it reveals parasites where induced malaria is being followed or in areas severely endemic for malaria. If well-made, well-stained, competently examined thick blood films are consistently negative in the absence of antipaludic medication, search should be made for an alternative diagnosis, excluding infections which may be recognized serologically, bacteriologically, or otherwise, before making an elective assumption regarding malarial etiology.

These fallacies and truths should be emphasized in medical and public health schools. They should be discussed in medical societies and during individual contacts with doctors. Members of the National Malaria Society engaging in these activities will be assisting materially in preventing spurious malaria reporting. They can aid similarly in promoting the currently intensified program of physician information concerning national malaria eradication, its importance to the country, and the practitioner's role in achieving it.

Endemic malaria is not yet eliminated from the United States. It may be years before this is accomplished—but it is of paramount importance to the Federal, State, and local agencies supporting the program to know if and when the goal is reached. It is recognized that the problem is an exceedingly complicated one, requiring broad perspective and intellectual honesty in the fields of medical epidemiology, medical entomology, vector-control engineering, and perhaps other phases of malariology. It would seem desirable that the formulations of conditions to be fulfilled should be made by parties not concerned primarily in the operation of the program. It is believed that the most authoritative, detached, and unbiased approach to this proposition can be made by the National Malaria Society. Accordingly, this organization has been officially requested by the Communicable Disease Center of the Public Health Service to take the steps necessary to provide a gauge by which eradication accomplishment can be measured.

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OBSERVATIONS OF THE PERSISTENCE OF *PLASMODIUM* INFECTIONS IN *ANOPHELES* MOSQUITOES IN AN AREA OF LOW OBSERVED HUMAN MALARIA PARASITEMIA IN SOUTH CAROLINA

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Since October 1944, a Negro population of approximately 2,000 persons in an endemic malarious area on Potato Creek in Clarendon County, South Carolina, has been surveyed each month for malaria, using thick blood films, to determine the incidence of malaria parasitemia. A continuous decline shown in the observed monthly parasitemia rates from 38.2 per cent positive in October 1944 to 0.0 per cent in September 1946 (Weathersbee and Frohne, 1948) has resulted in considerable speculation as to whether or not transmission of malaria continues to occur within the area under consideration. It is desirable to know the status of malaria transmission in association with the concerted and evidently successful effort being made to reduce malaria by the use of DDT as a residual spray in the houses. Accordingly, a program of dissection of *Anopheles quadrimaculatus* was undertaken. Moreover, since *A. crucians* (*crucians*) is naturally infected with an undetermined species of *Plasmodium* in this area (Sabrosky *et al.*, 1946), a parallel program of dissection of *crucians* was conducted to permit comparison of local human and anopheline malaria infections. The persistence of *Plasmodium* infections actually found in both local species of *Anopheles* was in marked contrast to the apparent disappearance of malaria parasitemia in humans. This paper discusses possible explanations for this anomalous lack of agreement between the two measures of malaria incidence, and the nature of the sporozoites found in *crucians*.

In the southern United States, the occurrence of sporozoite-positive *A. quadrimaculatus* mosquitoes in demonstrable numbers is usually associated with human parasitemia rates that are of such magnitude as to be readily determinable. Conversely, when human parasitemia rates are low, it appears that the occurrence of sporozoite-positive mosquitoes is difficult to demonstrate, and the recurrence of latent infections provides the only evidence of earlier transmission. In the present study, however, human parasitemia virtually disappeared in 1946, yet relatively high sporozoite rates in *A. quadrimaculatus* have persisted through 1947 and 1948. Moreover, during the same period, sporozoite rates for *A. crucians* were one and a half to two times as high as for *quadrimaculatus*, although *crucians* is not generally regarded as a natural vector of human malaria.

A possible explanation for this anomaly is suggested by the findings of another study conducted in this area (Young *et al.*, 1948) in which the infectivity to *A. quadrimaculatus* of persons with a previously positive blood film was tested. It was

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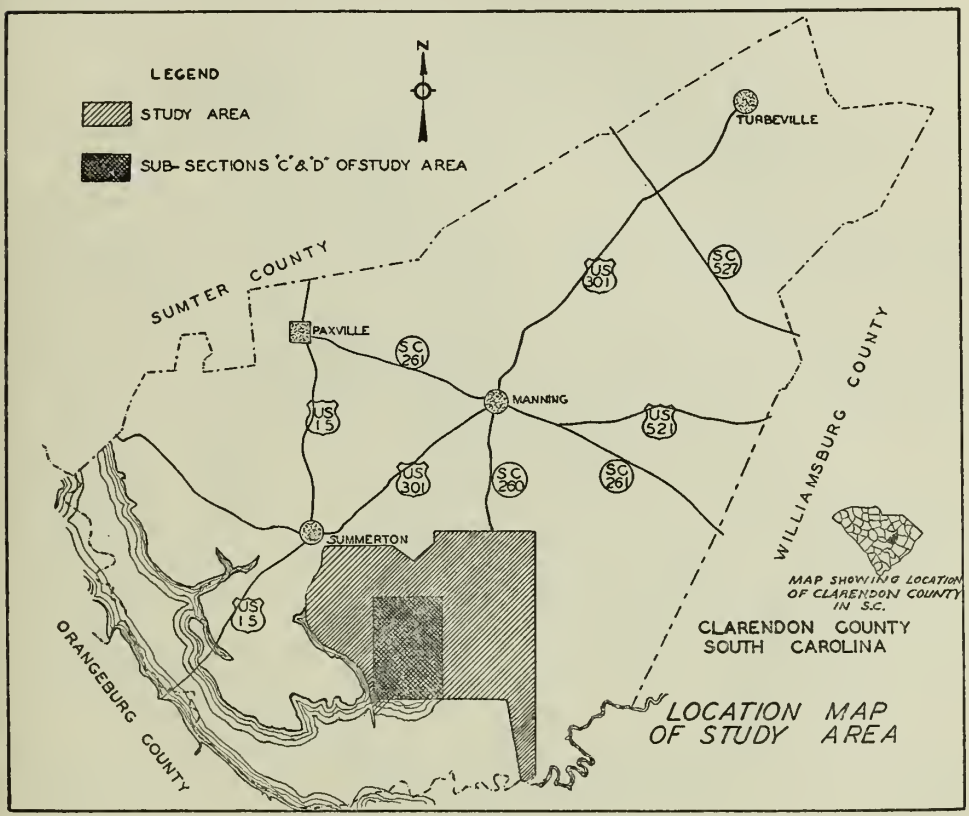
found that members of this species of mosquito were infected with *Plasmodium falciparum* and *P. vivax* by patients having less than 10 gametocytes per cmm. of blood. Some patients with no demonstrable gametocytes infected *A. quadrimaculatus* with *P. falciparum*. Favorable conditions for transmission, as indicated by the development of *Plasmodium* within the mosquito host, thus undoubtedly occur in this area. For instance, in 1945 and 1946, Weathersbee and Frohne (1948) were unable to establish a minimum population density for *quadrimaculatus* below which sporozoite-positive individuals could not be found.



Map 1

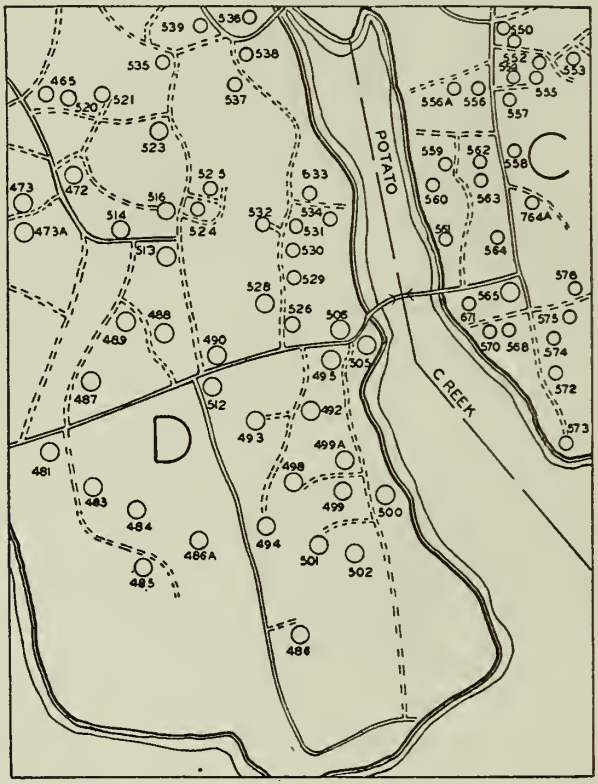
HUMAN MALARIA

Nearly three-fourths of the recognized infections in the human population of about two thousand within the study area have come from approximately six hundred people living in an area of roughly 13 square miles adjacent to the Santee Reservoir and Potato Creek. This area is comprised of sections designated as "C" and "D" on the accompanying maps 1 and 2. In table I, parasitemia data for this area for the 51 months, October 1944 through December 1948, are shown. A steady decrease in malaria prevalence was evidenced, except for a small increase in the fall of 1945. A rapid decline through 1946 was not interrupted by an autumn rise. The human

parasitemia rates for 1947 are insignificant, ranging from 0.0 per cent in January and July to 0.7 per cent in October. In 1948, only two cases of human malaria were found in sections "C" and "D", one in January and the other in October. Corresponding 1948 data from the study area as a whole show only 10 positive films, including four probable *P. malariae* recurrences.

ANOPHELES DISSECTIONS AND DENSITIES

Stables throughout all of the study area have been designated as both "dissection" and "index" (population indicator) stations. About half of these stations are in sec-



Map 2

tions "C" and "D", and more than two-thirds of the positive mosquitoes reported herein were collected there. These stables were visited weekly, counts were made of all *Anopheles* present, and dissection material was collected. Chart I represents the average number of females per station per week for the two common *Anopheles* during 1947 and 1948, and the occurrence of each gland-positive individual is indicated by an asterisk. These positives are tabulated in tables II and III. It is worthy of note that the occurrence of positive mosquitoes of both species tended to follow peaks in their density levels within one to three weeks. It is not intended in chart I to compare densities of *A. quadrimaculatus* and *A. crucians*, but rather to present the densities of each species through two successive seasons.

The dissection of more than 50,000 *Anopheles, crucians* and *quadrimaculatus*, from the whole study area during the past 4 years justifies confidence that the seasonal sporozoite rates are based on sufficient numbers to be significant.

Anopheles quadrimaculatus: Dissections were made from approximately equal *A quadrimaculatus* densities in 1945, 1946 (Weathersbee and Frohne, 1948), and 1948; however, 1947 was a season of low numbers. Moreover, large numbers of this species were dissected in each of these years, except 1947 when a sporozoite rate of 0.09 per cent was determined by the dissection of only 2,125 mosquitoes.

TABLE I

Results of Blood Film Examinations, Sections "C" and "D" October 1944–December 1948

	MONTHS												
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1944													
No. exam.										469	392	459	1310
No. positive										179	106	80	365
Rate (Per cent)										38.2	27.0	17.4	27.9
1945													
No. exam.	534	604	613	622	616	608	586	583	576	576	600	587	7105
No. positive	64	48	76	85	66	57	38	30	54	42	58	38	656
Rate (Per cent)	12.0	7.9	12.4	13.7	10.7	9.4	6.5	5.1	9.4	7.3	9.7	6.5	9.2
1946													
No. exam.	562	570	546	562	588	582	591	591	588	579	572	564	6895
No. positive	36	21	11	5	7	17	4	6	0	1	1	1	110
Rate (Per cent)	6.4	3.7	2.0	0.89	1.2	2.9	0.68	1.0	0.0	0.17	0.17	0.18	1.6
1947													
No. exam.	571	567	574	575	591	597	588	580	611	601	576	443	6874
No. positive	0	3	1	2	1	2	0	1	2	4	3	1	20
Rate (Per cent)	0.0	0.53	0.17	0.35	0.17	0.34	0.0	0.17	0.33	0.67	0.52	0.23	0.3
1948													
No. exam.	527	571	583	589	588	586	587	590	599	565	560	528	6873
No. positive	1	0	0	0	0	0	0	0	0	1	0	0	2
Rate (Per cent)	0.19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.18	0.0	0.0	0.03

Of special interest is the apparent reducing effect of DDT house spraying on human malaria rates and its failure to effect a commensurate reduction in mosquito infections. In 1945 only one-half of the study area was sprayed, but the sporozoite rates for *A. quadrimaculatus* in the sprayed and the unsprayed sections were not significantly different for that year (sprayed—0.14 per cent; unsprayed—0.19 per cent positive). The spectacular difference rests in the drop of average number of cases of malaria from 54.6 per month in 1945 to 9.2 in 1946. At the same time sporozoite rates in *A. quadrimaculatus* decreased from 0.17 per cent in 1945 to 0.07 per cent in 1946. Similar comparisons are not made with the 1947 data because of that year's low mosquito densities and the small number of mosquitoes dissected. However, the

contrast between 1945 and 1948 is startling. The year 1948, with two demonstrated cases, gives an annual human parasitemia rate of 0.029 per cent of persons examined; whereas the year 1945, with 656 demonstrated cases, produces a rate of 9.23 per cent of persons examined. This represents a ratio of 318 in 1945 to 1 in 1948. Yet, the sporozoite-positive *A. quadrimaculatus* rate for 1948 was 0.04 per cent, or approximately one-fourth as high as the 1945 rate.

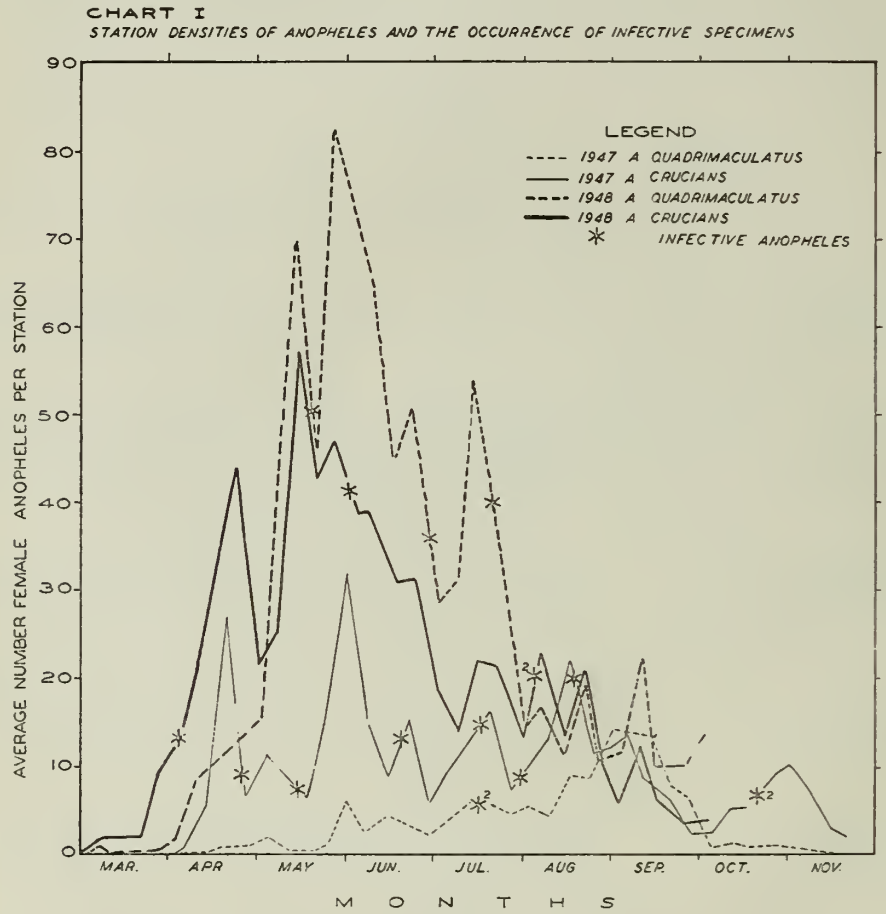


Chart 1

Anopheles crucians: The adults of this species occur in high densities during April–October, inclusive. Sporozoite-positive individuals of this species have been found in each of these months and also in November.

Simmons (1941) pointed out that too few dissections of the *crucians* species-complex had been done to judge the sanitary importance of any of the three species. As a matter of fact, it is believed that more dissections (well over 10,000) of the typical form (*A. crucians crucians*) have been made in the present study than the total for all three "*crucians*" species dissected heretofore. A principal finding in this area has been

the occurrence of rather consistently higher sporozoite rates in *crucians* than in *quadrimaculatus*. A comparison of 1947 and 1948 rates for *crucians* (table III), 0.14 per cent and 0.06 per cent, respectively, shows a drop similar to that observed in *quadrimaculatus*. Since *crucians* is considered not to become infected when gameto-

TABLE II

Summary of Dissections of Anopheles quadrimaculatus *from the Entire Study Area during 1947 and 1948*

1947				1948			
Month	Total Dissected	Total Infective	Rate	Month	Total Dissected	Total Infective	Rate
			Per cent				*Per cent*
Feb.–March	0	0	—	Feb.–March	8	0	—
April	56	0	—	April	454	0	—
May	230	0	—	May	1,326	1	0.08
June	374	0	—	June	1,323	0	—
July	512	2	0.39	July	1,366	2	0.15
August	346	0	—	August	1,421	0	—
September	523	0	—	September	1,904	0	—
October	74	0	—	October	656	0	—
November	10	0	—	November	6	0	—
Totals	2,125	2	0.09	Totals	8,464	3	0.04

TABLE III

Summary of Dissections of Anopheles crucians *from the Entire Study Area during 1947 and 1948*

1947				1948			
Month	Total Dissected	Total Infective	Rate	Month	Total Dissected	Total Infective	Rate
			Per cent				*Per cent*
Feb.–March	4	0	—	Feb.–March	422	0	—
April	605	1	0.16	April	1,583	1	0.06
May	1,204	1	0.08	May	1,165	0	—
June	878	1	0.11	June	992	1	0.10
July	578	1	0.17	July	1,026	0	—
August	547	1	0.18	August	1,242	2	0.16
September	318	0	—	September	700	0	—
October	498	2	0.40	October	540	0	—
November	506	0	—	November	714	1	0.14
Totals	5,138	7	0.14	Totals	8,384	5	0.06

cyte density is low (Simmons, 1941), the infections encountered in this species, if of human origin, are noteworthy. Its habits of biting and remaining outside of houses would seem to account for the occurrence of *Plasmodium* in an area where the houses were sprayed. In the following paragraphs on individual positive dissections, data

are presented which indicate that sporozoites found in *crucians* and *quadrimaculatus* probably include the same species of *Plasmodium*.

PLASMODIUM-POSITIVE STATIONS

About one-fourth (10) of the dissection stations furnished gland-infected *Anopheles* in 1947 and 1948, and one station yielded three positives each year. Table IV presents the details of human malaria information on the members of the households at stations in which sporozoite-positive mosquitoes were found.

What appears to be significant epidemiological evidence bearing on the species of sporozoites found in *A. quadrimaculatus* and *A. crucians* in a malarious neighborhood (stations 484, 485, and 486) in section "D" is discussed below.

The homes of two large, malarious Negro families with histories of *P. falciparum* are located at stations 484 and 486. Three members of each of these families at 484 and 486 were found positive in 1946. However, only one person, 7-year old C. T. at station 484, was again found positive in August and September 1947. His positive smear was the only instance of human parasitemia found in August and one of two found in September for the entire "C" and "D" population of about six hundred. On July 17, 1947, a gland-positive *A. quadrimaculatus* was taken from the stable at station 484. Moreover, the only other positive *A. quadrimaculatus* taken during 1947 was also collected on July 17 from nearby station 486. In addition, a sporozoite-positive *A. crucians* was secured from station 484 (C. T.'s stable) on June 20, 1947. A positive *A. crucians* was taken from station 486 on May 15 and another on July 17. Thus, from these two stations located within one-half mile of each other, and in one of which a positive malaria smear was found in August and September, two infected *A. quadrimaculatus* and three infected *A. crucians* were collected during May, June and July. Furthermore, of this total of five positive mosquitoes, two *A. quadrimaculatus* and one *A. crucians* were taken on a single day, July 17, from these stations. In 1947, then, these two neighboring stations produced not merely all the positive *A. quadrimaculatus* (two) of 2,125 dissected, and both on the same day, but also three of the seven positive *A. crucians* of 5,138 *crucians* dissected.

In 1948 the 484–486 neighborhood was watched with intense interest. As already noted, no human parasitemia was detected. However, on April 2 the first positive *Anopheles* of the season, a *crucians*, was collected from the stable at 486. On May 19 a gland-positive *quadrimaculatus* came from vacant station 485, which was nearby. On June 30 a positive *quadrimaculatus* was collected at 486; and on August 4 a second positive *crucians* was taken from the same station. In 1948 this neighborhood yielded 50 per cent of the positive *Anopheles* found, but these three stations and station 483 produced only 12.6 per cent of the mosquitoes dissected. As in the preceding year, both *quadrimaculatus* and *crucians* positives had appeared at an unusually high rate in the same small neighborhood. In 1948, however, the positive mosquitoes occurred in the apparent absence of human malaria.

The majority of the mosquito-positive stations, unlike houses 484 and 486, are scattered and each has furnished a single infected mosquito. Some have had a history of human malaria in the last three years, while others have not (table IV). It seems futile to speculate over whether or not any of these isolated infected *Anopheles* had

TABLE IV

Anopheles *Dissections from Positive Stations, 1947–1948, and Malaria History of the Household January 1946–November 1948*

Station E-369. Malaria History: Negative for all 31 months checked; 3 members; one *A. crucians* infective October 20, 1947.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	86	303	88	86	30	11	124	118
No. Positive	0	0	0	0	0	0	0	1	0
Rate (Per cent)	—	—	—	—	—	—	—	0.8	—
Cum. Tot. Diss.	0	86	389	477	563	593	604	728	846
Cum. No. Pos.	0	0	0	0	0	0	0	1	1
Cum. Rate (Per cent)	—	—	—	—	—	—	—	0.1	0.1

Station 484. Malaria History: Checked all 33 months; 14 members; showing—*P. falciparum*, members 10 and 13 in January 1946; *P. falciparum*, members 10 and 12 in February 1946; *P. falciparum*, member 10, in August and September 1947; one *A. quadrimaculatus* July 17, 1947, and one *A. crucians* infective June 1947.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	23	45	129	48	79	15	21	14
No. Positive	0	0	0	1	0	0	0	0	0
Rate (Per cent)	—	—	—	0.8	—	—	—	—	—
Cum. Tot. Diss.	0	23	68	197	245	324	339	360	374
Cum. No. Pos.	0	0	0	1	1	1	1	1	1
Cum. Rate (Per cent)	—	—	—	0.5	0.4	0.3	0.3	0.3	0.3

A. quadrimaculatus, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	6	1	26	27	44	54	7	0
No. Positive	0	0	0	0	1	0	0	0	0
Rate (Per cent)	—	—	—	—	3.7	—	—	—	—
Cum. Tot. Diss.	0	6	7	33	60	104	158	165	165
Cum. No. Pos.	0	0	0	0	1	1	1	1	1
Cum. Rate (Per cent)	—	—	—	—	1.7	1.0	0.6	0.6	0.6

TABLE IV—*Continued*

Station 485. Malaria History: Checked all 24 months 1946–1947; 3 members (uninhabited 1948); showing *P. falciparum*, member 1 in January 1946; one *A. quadrimaculatus* infective May 19, 1948.

A. quadrimaculatus, 1948

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	0	8	96	8	0	0	0	0
No. Positive	0	0	1	0	0	0	0	0	0
Rate (Per cent)	—	—	12.5	—	—	—	—	—	—
Cum. Tot. Diss.	0	0	8	104	112	112	112	112	112
Cum. No. Pos.	0	0	1	1	1	1	1	1	1
Cum. Rate (Per cent)	—	—	12.5	1.0	0.9	0.9	0.9	0.9	0.9

Station 486. Malaria History: Checked all 33 months, 15 members; showing—*P. falciparum* members 10 and 12, January 1946; *P. falciparum*, members 11 and 12, August 1946; two *A. crucians* infective, May 15, 1947, and July 17, 1947; two *A. quadrimaculatus* infective, July 17, 1947, and June 30, 1948.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	83	204	177	203	137	62	128	77
No. Positive	0	0	1	0	1	0	0	0	0
Rate (Per cent)	—	—	0.5	—	0.5	—	—	—	—
Cum. Tot. Diss.	0	83	287	464	667	804	866	994	1,071
Cum. No. Pos.	0	0	1	1	2	2	2	2	2
Cum. Rate (Per cent)	—	—	0.3	0.2	0.3	0.2	0.2	0.2	0.2

A. quadrimaculatus, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	33	49	61	115	68	42	10	2
No. Positive	0	0	0	0	1	0	0	0	0
Rate (Per cent)	—	—	—	—	0.9	—	—	—	—
Cum. Tot. Diss.	0	33	82	143	258	326	368	378	380
Cum. No. Poss.	0	0	0	0	1	1	1	1	1
Cum. Rate (Per cent)	—	—	—	—	0.4	0.3	0.3	0.3	0.3

A. crucians, 1948

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	25	49	46	49	112	186	112	283	29
No. Positive	0	1	0	0	0	1	0	0	0
Rate (Per cent)	—	2.0	—	—	—	0.5	—	—	—
Cum. Tot. Diss.	25	74	120	169	281	467	579	862	891
Cum. No. Pos.	0	1	1	1	1	2	2	2	2
Cum. Rate (Per cent)	—	1.4	0.9	0.6	0.4	0.4	0.3	0.2	0.2

TABLE IV—*Continued*

A. quadrimaculatus, 1948

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	2	71	38	58	51	144	62	36	0
No. Positive	0	0	0	1	0	0	0	0	0
Rate (Per cent)	—	—	—	1.7	—	—	—	—	—
Cum. Tot. Diss.	2	73	111	169	220	364	426	462	462
Cum. No. Pos.	0	0	0	1	1	1	1	1	1
Cum. Rate (Per cent)	—	—	—	0.6	0.5	0.3	0.2	0.2	0.2

Station 503. Malaria History: Negative for all 33 months: 8 members; one *A. crucians* positive July 30, 1947.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	4	21	35	109	48	10	40	32	76
No. Positive	0	0	0	0	1	0	0	0	0
Rate (Per cent)	—	—	—	—	2.1	—	—	—	—
Cum. Tot. Diss.	4	25	60	169	217	227	267	299	375
Cum. No. Pos.	0	0	0	0	0	1	1	1	1
Cum. Rate (Per cent)	—	—	—	—	0.5	0.4	0.4	0.3	0.3

Station 562. Malaria History: All 33 months checked; 11 members; showing—*P. falciparum*, members 1, 8 and 10, February 1946; *P. falciparum*, member 9, May 1947; *P. falciparum*, member 10, June 1947 and November 1947; one *A. crucians* positive April 29, 1947.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	5	10	4	1	3	2	0	0
No. Positive	0	1	0	0	0	0	0	0	0
Rate (Per cent)	—	20.0	—	—	—	—	—	—	—
Cum. Tot. Diss.	0	5	15	19	20	23	25	25	25
Cum. No. Pos.	0	1	1	1	1	1	1	1	1
Cum. Rate (Per cent)	—	20.0	6.7	5.3	5.0	4.3	4.0	4.0	4.0

Station 618. Malaria History: Checked all 33 months; 5 members; showing—*P. malariae*, member 8 in April 1946; one *A. crucians* positive August 20, 1947.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	3	2	2	2	2	2	0	0
No. Positive	0	0	0	0	0	1	0	0	0
Rate (Per cent)	—	—	—	—	—	50.0	—	—	—
Cum. Tot. Diss.	0	3	5	7	9	11	13	13	13
Cum. No. Pos.	0	0	0	0	0	1	1	1	1
Cum. Rate (Per cent)	—	—	—	—	—	9.1	7.7	7.7	7.7

TABLE IV—*Continued*

Station 783. Malaria History: Checked only 9 times, viz. January 1946, 1947; April 1946, 1947, 1948; July 1946, 1947; October 1946, 1947; 10 members; showing—*P. falciparum*, member 11, July 1946; one *A. crucians* positive June 1, 1948 (station not dissected in 1947).

A. crucians, 1948

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	8	90	77	53	107	132	70	53	144
No. Positive	0	0	0	1	0	0	0	0	1
Rate (Per cent)	—	—	—	1.9	—	—	—	—	0.7
Cum. Tot. Diss.	8	98	175	228	335	467	537	590	734
Cum. No. Pos.	0	0	0	1	1	1	1	1	2
Cum. Rate (Per cent)	—	—	—	0.4	0.3	0.2	0.2	0.2	0.3

Station 858. Malaria History: Checked 31 months (omitting February and March, 1946); 9 members; showing no positive slide; 1 *A. crucians* positive October 20, 1947; 1 *A. quadrimaculatus* positive July 19, 1948.

A. crucians, 1947

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	2	0	6	2	1	9	13	50
No. Positive	0	0	0	0	0	0	0	1	0
Rate (Per cent)	—	—	—	—	—	—	—	7.6	—
Cum. Tot. Diss.	0	2	2	8	10	11	20	33	83
Cum. No. Pos.	0	0	0	0	0	0	0	1	1
Cum. Rate (Per cent)	—	—	—	—	—	—	—	3.0	1.2

A. quadrimaculatus, 1948

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	0	33	102	48	132	130	49	13	0
No. Positive	0	0	0	0	1	0	0	0	0
Rate (Per cent)	—	—	—	—	0.8	—	—	—	—
Cum. Tot. Diss.	0	33	135	183	315	445	494	507	507
Cum. No. Pos.	0	0	0	0	1	1	1	1	1
Cum. Rate (Per cent)	—	—	—	—	0.3	0.2	0.2	0.2	0.2

Station 822. Malaria History: Checked 30 months (omitting February and March 1946, August 1948); 6 members; showing no positive slide; one *A. crucians* positive August 2, 1948.

A. crucians, 1948

	FEB.–MARCH	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.
Total Dissected	41	73	87	45	124	99	72	13	197
No. Positive	0	0	0	0	0	1	0	0	0
Rate (Per cent)	—	—	—	—	—	1.0	—	—	—
Cum. Tot. Dis.	41	114	201	246	370	469	541	554	751
Cum. No. Pos.	0	0	0	0	0	1	1	1	1
Cum. Rate (Per cent)	—	—	—	—	—	0.2	0.2	0.2	0.1

access to the few known instances of human parasitemia. It seems reasonable, however, that another much larger and more widely distributed source of infection than the fraction of a per cent of recognized human parasitemia infected most of them. There is the possibility that mosquitoes may have become infected with parasites occurring in humans at densities so low as to be missed altogether by ordinary survey examinations. Another possibility is that the infection may be that of an unknown animal malaria. Although seven infected *Anopheles* were taken during 6 months of 1948, not one instance of human parasitemia was found in the 12,000 blood films from about 2,000 persons within the study area.

DISCUSSION

Three findings in these studies are so unusual as to call for explanation or at least comment: (1) A sporozoite rate in *A. crucians* higher than that of *quadrimaculatus;* (2) Presence of sporozoite-positive *Anopheles* in an area where the houses have been sprayed with DDT; (3) Relatively high sporozoite rates in *Anopheles* after the virtual disappearance of demonstrable human parasitemia.

Relatively high sporozoite rates in *crucians* suggest the possibility of animal malaria. Modest attempts to identify the sporozoites have only shown that they are within the size range of *P. falciparum* and *vivax*, averaging 8.4 microns between the apices. Feeding wild *crucians*, in a relatively few instances, on human paretics has failed to induce malaria. The *crucians* used were collected from stations which were recently the source of an infective *crucians*. Injection of sporozoites from glands of nine *crucians* into canaries has caused no avian malaria infections; sub-inoculations from these canaries into clean canaries likewise produced no infections. In an indirect approach, different canaries were inoculated with *P. relictum*, *elongatum*, and *circumflexum* and, from time to time, when gametocytes and exflagellation indicated infectivity, 26 lots of reared *crucians* were fed on them. A total of 238 *crucians* in these 26 lots which survived until dissection after 2 weeks were all negative. The data are inadequate for a conclusion on the provenience, human or animal, of the sporozoites in *crucians*. However, this does not alter the concept that sporozoites in wild *Anopheles* in the United States may be human malaria. The infective neighborhood case history and the similarity in size of the undetermined sporozoites to *falciparum* sporozoites tend to support it. Postulation of animal malaria and a concerted investigation of possible vertebrate hosts might afford data which would help to establish the identity of the sporozoites being encountered in association with so little or no patent human malaria.

More intensive search for human malaria persisting in the area might be informative. Human parasitemias have commonly had to be diagnosed on "rare" or even "very rare" parasites. Further studies like that of Young *et al.* (1948) feeding *Anopheles* on persons with such low-density parasitemias are needed.

The presence of sporozoite-positive *Anopheles* in this sprayed area is an interesting fact. Residual spraying has with few exceptions been shown to have kept these houses mosquito-free in the daytime. Whether or not this has reduced the sporozoite rates of *Anopheles* has not been determined. We do know however that in 1945 the unsprayed (control) half of the study area had an insignificantly higher rate, and

whether or not its influence on rates for *crucians* would be comparable to that for *quadrimaculatus* has not been determined. In this connection it might be well to call attention to Boyd's (1930) observation "that the contraction of infection outside of dwellings is not unlikely or even rare."

As stated in the introduction, the persistence of relatively high sporozoite rates in *Anopheles* where monthly blood films of all the human population indicate little or no malaria is a phenomenon deserving further study. The strain of *P. falciparum* in the study area is atypical. The data of Young *et al.*, (1948) showed it to be capable of infecting *quadrimaculatus* at extremely low gametocyte densities. Reider and McDaniel (in press) found its infections persisted for unusually long periods. If the infections in *crucians* can be identified as human malaria, the apparently unusual susceptibility of this species of *Anopheles* may prove to be another aberrant characteristic of the *falciparum* malaria encountered within the area under study.

SUMMARY AND CONCLUSIONS

1. Data are presented on malaria incidence as determined by monthly blood films for a period of 51 months in a Negro population of about six hundred. The parasitemia rate dropped from 38.2 per cent in October 1944 to 0.0 per cent in 1946, and has remained low during 1947 and 1948.

2. The results of dissection of 33,593 *A. quadrimaculatus* in 1945 and 1946 and of 10,589 in 1947 and 1948 show a generally parallel drop in the annual sporozoite (gland-positive) rates. Rates of the four successive years are: 0.17, 0.07, 0.09, and 0.04 per cent, respectively.

3. Similarly, the sporozoite rates of 5,138 *A. crucians* dissected in 1947 and of 8,384 in 1948 were 0.14 and 0.06 per cent, respectively.

4. Densities for both species of *Anopheles* were determined from weekly averages of dissection station counts. There were low densities in 1947, but *A. crucians* densities were not so greatly depressed as those of *A. quadrimaculatus*.

5. During 1947 and 1948 when human parasitemia rates were insignificant, four individual stations yielded five sporozoite-positive *quadrimaculatus*, and nine stations furnished 12 infected *crucians*. In four instances gland-positive mosquitoes of both species came from the same stations. Positive *crucians* were found in all *quadrimaculatus*-positive stations except one vacant house from which less than two hundred *crucians* were dissected in two years.

6. A formerly malarious neighborhood of four dissection stations produced the two infective *quadrimaculatus* of 1947 and three of the seven infective *crucians*. In 1948 the same small neighborhood without any demonstrable human parasitemia during all 12 months, nevertheless furnished 50 per cent of the infective *Anopheles* found, though only 12.6 per cent of the mosquitoes dissected came from there.

7. In the study area the residual spraying of dwellings with DDT was carried on from 1945 through 1948. During that period human malaria parasitemia decreased in the ratio of 318 to 1. In spite of this remarkable reduction in parasitemia, and doubt regarding any recent new infections, the conclusion is reached that a minor amount of malaria transmission may be occurring in the study area for the following reasons: (1) Sporozoite-positive anophelines were present at specific locations in

association with human parasitemia. Further investigations to determine the nature of these sporozoite infections is necessary before the definite conclusion can be drawn that they are not of human origin. (2) *P. falciparum* infections in the human host are atypical. These usually are asymptomatic, persist for long priods, and are highly infective to *Anopheles* even when gametocytes rates are extremely low. Thus, it is possible that a higher percentage of the human population is infective to *Anopheles* than is indicated by the monthly human parasitemia rates.

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SUMARIO Y CONCLUSIONES

1. Se presentan datos acerca de incidencia de malaria determinados por exámenes mensuales de frotes durante un período de 51 meses en una población negra de alrededor de seiscientos habitantes. El índice parasitario bajó de 38,2 por ciento en octubre de 1944 a 0,0 en 1946 habiendo permanecido bajo durante 1947 y 1948.

2. Los resultados de la disección de 33.593 *A. quadrimaculatus* en 1945 y 1946 y de 10.589 en 1947 y 1948 muestran una rebaja paralela en los índices esporozoíticos anuales. Los índices en los cuatro años sucesivos fueron: 0,17, 0,07, 0,09 y 0,04 por ciento, respectivamente.

3. Similarmente los índices esporozoíticos de 5.138 *A. crucians* disecados en 1947 y de 8.384 en 1948 fueron de 0,14 y 0,06 por ciento, respectivamente.

4. Las densidades para ambas especies de *Anopheles* fueron determinadas a base de la media semanal de los contajes en las estaciones de captura. Hubo densidades bajas en 1947 pero el descenso en las densidades de *A. crucians* fué menos pronunciado que el de *A. quadrimaculatus*.

5. Durante 1947 y 1948 en que los índices parasitarios fueron insignificantes, cuatro estaciones de captura produjeron cinco *quadrimaculatus* positivos a esporozoítos y nueve estaciones produjeron 12 *crucians* infectados. En cuatro ocasiones mosquitos de ambas especies con las glándulas infectadas procedían de las mismas estaciones de captura. *Crucians* positivos fueron hallados en todas las estaciones de captura con *quadrimaculatus* positivos, a excepción de una casa desocupada donde menos de 200 *crucians* fueron disecados en dos años.

6. Cuatro estaciones de captura localizadas en un vecindario antiguamente malárico produjeron los dos *quadrimaculatus* infectantes de 1947 y tres de los siete *crucians* infectantes del mismo año. En 1948 el mismo vecindario a pesar de no acusar parasitemia entre sus habitantes durante los doce meses produjo el 50 por ciento de los *Anopheles* infectantes encontrados, no obstante que solo el 12,6 por ciento de los mosquitos eran de su procedencia.

7. En el área estudiada se rociaron las casas con DDT residual desde 1945 hasta 1948. Durante este período la parasitemia bajó en la proporción de 318 a 1. A pesar de esta notable reducción en la parasitemia y de no haber recientes primoinfecciones se concluye que pudiera ocurrir una pequeña transmisión en el área estudiada, debido a las siguientes razones: (1) Anophelinos positivos a esporozoítos estuvieron presentes en determinados puntos en asociación con parasitemia humana. Más investigaciones para determinar la naturaleza de estas infecciones esporozoíticas son necesarias antes de llegar a la conclusión de que no son de origen humano. (2) Las infecciones de *A. falciparum* en el hombre fueron atípicas. Estas generalmente son asintomáticas, persisten por largo tiempo y son altamente infectantes a los *Anopheles* aún cuando las infecciones gametocíticas sean extremadamente bajas. Por consiguiente, es posible, que un porcentaje mayor de la población humana sea infectante a los *Anopheles* que la indicada por los índices parasitarios mensuales.

A REPORT ON ONE YEAR'S FIELD TRIAL OF CHLORGUANIDE (PALUDRINE) AS A SUPPRESSIVE AND AS A THERAPEUTIC AGENT IN SOUTHERN TAIWAN (FORMOSA)[1]

ROBERT B. WATSON, J. HARLAND PAUL AND K. C. LIANG

In the course of research in the United Kingdom and the United States during recent war years to discover antimalarial drugs at least equal to quinine and quinacrine (atabrine) many thousands of compounds were synthesized and tested. Few proved to be of practical use and only chlorguanide[2] was available in substantial supply in 1946. The drugs which had shown greatest promise had not been studied extensively under practical field conditions. At least three agents with practical potentialities as causal prophylactics and as schizontocides were found.

Fairley (1946) showed in 1946 that chlorguanide was effective against the preerythrocytic stages of *Plasmodium falciparum*, less so against these forms of *Plasmodium vivax;* and that the drug destroyed erythrocytic forms of *P. falciparum* and *P. vivax*. Chlorguanide was proclaimed a complete causal prophylactic against falciparum malaria, a partial causal prophylactic against vivax malaria and a powerful schizontocide. By 1947 the drug was successfully employed for clinical treatment of malaria throughout the world.

Early in 1947 sufficient supplies of chlorguanide and of two 4-aminoquinoline compounds were on hand to allow trial of these drugs in malaria control work in Formosa. The objective of the present study was to test the efficiency of chlorguanide when used in minimal doses at weekly intervals under Formosan field conditions. Secondarily we wished to learn the practical usefulness of chlorguanide.

STUDY CONDITIONS

Under the Japanese government of Formosa, the colonial malaria control program was supervised by the police department and consisted principally of the administration of quinine. There were more than 200 malaria treatment centers on the island, each equipped to examine blood films and to dispense quinine. Persons whose blood was found to contain malaria parasites were required to take 0.8 gm. quinine per day for five days. If parasitemia persisted, treatment was repeated until no more parasites could be found.

More than three million blood films were examined in 1944 and records are largely intact. Records for the Ch'ao Chow District treatment center, our principal field

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Taiwan Provincial Malaria Research Institute of the Taiwan Provincial Health Department in co-operation with the International Health Division of The Rockefeller Foundation.

[2] The official U. S. name for N_1-(p-chlorophenyl)-N_5-isopropyl biguanide. Synonyms are: paludrine, proguanil B. P., M. 4888, guanatol, drinupal, palusil, tirian (Cooper 1949). British-made paludrine was used in this work and was supplied gratis by the Imperial Chemical Industries (China) Ltd., through their Shanghai Office.

station in Formosa, show a mean annual rate of 6.34 per cent positive blood films for the period 1937–44, which is believed to be a gross underestimation of the prevalence of parasitemia for this period. Evidence that blackwater fever was present in the district during this period would suggest a higher transmission rate than records indicate. There is evidence also that the technician of the center had so little proficiency in finding parasites in thick films that underestimations amounted to as much as 50 per cent. Taking into account all other factors we believe that parasitemia rates for the district were two to three times higher than the rates compiled by the treatment center.

The locality selected for this study was the village San Hsing, situated about 1 kilometer from the town of Ch'ao Chow in Kao Hsiung Hsien (County), about 35 miles southeast of Kao Hsiung city (Takao), the main southern Formosa port and former Japanese naval base. Ch'ao Chow township comprises 13 villages and one small town with a total population of some 17,000 people. It is one of the more malarious parts of Formosa; out of a total average of 383 persons examined in nine malaria surveys in 1947, the range for all ages from plus 26 to minus 32. San Hsing is typical of most villages in southern Formosa. The inhabitants are of Chinese origin; their forebearers originally emigrated from eastern Fukien or Kwangtung Provinces. Evidence of the regimentation of the population under the Japanese government still remains. The people are used to obeying directives, which facilitated our studies. Approximately 70 per cent of the total population of 417 derives a living directly from farming. The village is immediately surrounded by irrigated rice fiends with numerous adjacent canals and irrigation ditches. Most houses are of bamboo wattle construction plastered with mud or lime mortar. A few families live in brick masonry houses. The bamboo houses have open windows and doors and offer no hindrance to the entry of mosquitoes. The inhabitants are not protected otherwise from mosquitoes: the very few bed-nets are of poor design, usually in bad condition and are not properly used. Domestic animals, mostly water buffalo and fowls, are quartered near the dwellings.

In this part of Formosa the rainy season, April to October, conforms with the hot season. However, there is no very great seasonal difference in temperature and the coldest months, December and January, are still warm enough to permit the development of parasites in the mosquito host. We believe, though we have not yet confirmed by mosquito dissection or otherwise, that malaria transmission may occur in Ch'ao Chow District throughout the entire year. Even in the dry season there is an abundance of water for breeding places of *Anopheles hyrcanus sinensis* and *Anopheles minimus*, the most important if not the only vectors among the 10 local anophelines. Water for irrigation is supplied by artesian wells that flow the year round, except at the height of the dry season. Bamboo pipes are driven into a ground water table 200 to 300 feet under the surface, which is under considerable hydrostatic pressure. The mosquito breeding areas in ditches, canals and fields result from agricultural practice rather than of rainfall. Consequently, as one would expect, there is a marked increase in anophelism during the period of preparation of rice fields and the cultivation of the two rice crops, in midwinter and late summer (see Figure 1).

METHODS

An initial malaria survey of the study population of San Hsing was made from June 26–30, 1947 (Table 1). The weight of each person was recorded, blood films were taken and a spleen examination was made. Except for weight records that were not retaken, identical surveys were made at intervals of six weeks until late December 1947, and every eight weeks thereafter.

When the examination of each person was completed in the first survey, he was required to take the suppressive dose of chlorguanide prescribed for his weight group. This dose was repeated at intervals of 7 days thereafter unless the recipient was already under chlorguanide treatment because of parasitemia. Persons who were found to have parasitemia at the first survey were given a therapeutic course of chlorguanide and another blood film was taken one week later. When the check film was taken, a suppressive dose was given; if the film was positive, another therapeutic

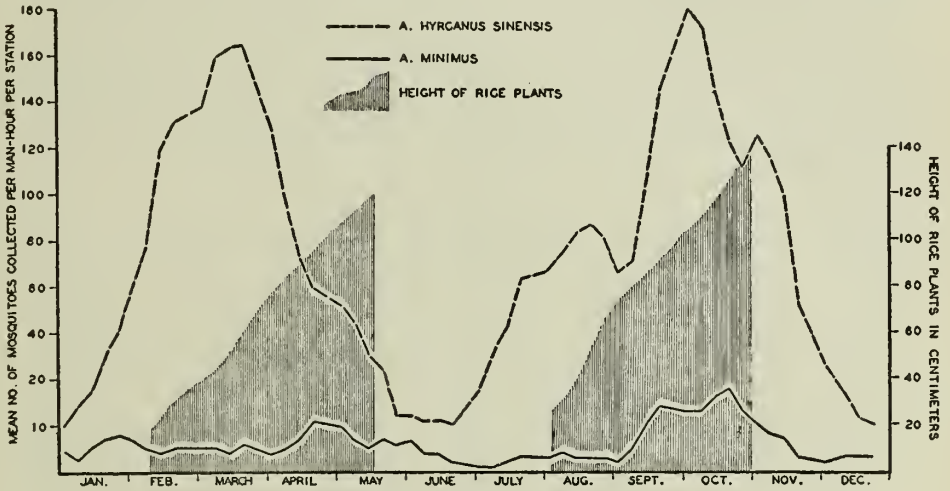

FIG. 1. Anophelism Curve

course was given. Because of the proximity of our laboratory to the study area, appropriate treatment could be given in a matter of hours after blood films were taken and examined.

The treatment schedules employed are shown in Tables 2 and 3. It will be noted that the dosage was not uniform throughout the study. The schedules initially employed doses that were low in comparison with usage elsewhere and the recommendations of the manufacturer of chlorguanide because there were no data on the use of the drug in Chinese and few data on its use in children in general. There was no way of knowing whether or not untoward effects might occur. One objective of the study was also to find the minimum dosage of chlorguanide which would give satisfactory suppression of malaria parasitemia. The maximum suppressive dose employed, 100 mgm. per week, was based upon Davey's (1946) opinion that this dose was likely to be efficient.

TABLE 1

Record of First Survey, June 26–30, 1947, San Hsing Village

Both Males & Females

CHINESE YEARS	TOTAL NO.	MEAN WGT.	SPLEEN SIZE						TOTAL ENLARGED SPLEENS	AVERAGE ENLARGED SPLEENS	SPLEEN RATE	BLOOD EXAMINATION					PARA. RATE
			0	1	2	3	4	5				Neg.	Positive				
													V.	F.	M.	Tol.	
		Kgms.									*per cent*						*per cent*
1 & 2	12	6.4	8	1	3	0	0	0	4	1.8	33	11	1	0	0	1	
3–5	40	11.8	11	6	17	6	0	0	29	2.0	73	21	12	5*	2*	19	
6–10	61	17.2	18	9	22	10	2	0	43	2.1	70	49	9	2	2	13	
11–15	48	26.9	3	13	22	10	0	0	45	1.9	94	37	3*	4*	4	11	
16–20	56	45.3	9	8	20	9	4	3	44	2.4	79	44	5	2	6	13	
21–30	75	50.1	17	13	27	13	2	0	55	2.1	73	68	2*	3*	2	7	
31–40	54	49.6	20	7	13	8	5	0	33	2.3	61	46	4	1	2	7	
41–50	22	50.0	11	2	3	3	1	1	10	2.6	45	18	1	0	2	3	
51 & Up	29	45.7	13	4	6	2	2	1	15	2.3	52	29	0	0	0	0	
All Ages	397		110	63	133	61	16	5	278	2.16	71.64	323	37	17	20	74	18.18

Note: The sign (*) indicates a mixed infection; this is counted as a single infection in the calculation of composite rates.

TABLE 2

Suppressive and Therapeutic Schedules Employed for the Administration of Chlorguanide. All Doses are Given in Grams

WEIGHT IN KILOGRAMS	SCHEDULE 1, 1ST TO 12TH WEEKS				SCHEDULE 2, 13TH TO 18TH WEEKS			
	Suppr. Dose	Therapeutic Dose			Suppr. Dose	Therapeutic Dose		
		Day 1	Day 2	Day 3		Day 1	Day 2	Day 3
9 or less	0.010	0.020	0.010	0.010	0.015	0.020	0.020	0.020
10 to 14	0.020	0.050	0.020	0.020	0.030	0.050	0.030	0.030
15 to 19	0.030	0.100	0.030	0.030	0.040	0.100	0.050	0.050
20 to 39	0.050	0.200	0.050	0.050	0.060	0.200	0.100	0.100
40 or more	0.100	0.300	0.200	0.100	0.100	0.300	0.300	0.200

AGE IN CHINESE YEARS	SCHEDULE 3, 31ST TO 55TH WEEKS			
	Suppr. Dose	Therapeutic Dose		
		Day 1	Day 2	Day 3
1 to 5	0.025	0.050	0.030	0.030
6 to 15	0.050	0.200	0.100	0.100
16 or Older	0.100	0.300	0.300	0.200

When no untoward effects occurred and there was increasing evidence of failure of suppression in the lower age groups, which received less than 2.0 mgm. per kilogram of weight, the dosage schedule was adjusted upward for children from the beginning of the 13th week. At the end of the 18th week suppressive treatment was

discontinued temporarily because of a low supply of chlorguanide, although proved cases of malaria continued to receive therapy. Suppressive treatment was resumed at the sixth survey, at the beginning of the 31st week, according to a schedule based on age since such a schedule was somewhat easier to administer than the discontinued one based on weight. Except for the lowest age group the two did not differ much, and the age-based schedule was continued to the end of the study.

Suppressive doses of chlorguanide were administered under supervision to insure the ingestion of the drug. Therapeutic doses were given under the supervision of the physician in charge, the daily dose being given as a single dose.

RESULTS

The results of the above-described administration of chlorguanide are given below in terms of general and species parasitemia levels in the study population and in

TABLE 3

Mean Weights of Population Studied and Approximate Dosage Employed, by Age Groups

(The therapeutic dosage recorded was computed from the mean daily dose of the three-day treatment period)

AGE IN CHINESE YEARS	MEAN WEIGHT KILOGRAMS			AVERAGE DOSE CHLORGUANIDE MGM./KGM. BODY WEIGHT					
	Males	Females	Both Sexes	Schedule 1		Schedule 2		Schedule 3	
				Suppr.	Therap.	Suppr.	Therap.	Suppr.	Therap.
1 & 2	6.7	6.1	6.4	1.6	2.1	2.3	2.6	3.9	5.7
3–5	11.3	12.3	11.8	1.0	2.5	2.5	3.1	2.1	3.1
6–10	17.0	17.4	17.2	1.7	3.1	2.3	3.9	1.7	3.9
11–15	26.5	27.4	26.9	1.9	3.7	2.2	5.0	1.9	5.0
16–20	44.6	46.0	45.3	2.2	4.4	2.2	5.9	2.2	5.9
21–30	52.1	48.1	50.1	2.0	4.0	2.0	5.3	2.0	5.3
31–40	52.2	47.0	49.6	2.0	4.7	2.0	5.4	2.0	5.7
41–50	53.8	46.3	50.0	2.0	4.0	2.0	5.3	2.0	5.3
50 & Over	49.3	42.2	45.7	2.2	4.4	2.2	5.8	2.2	5.8

terms of changes in spleen rates and size. No strictly comparable control studies could be made, because people will rarely submit to short interval surveys unless some inducement is offered in return. But certain comparisons are possible. These are comparison of the curve of parasitemia in the study village with the curve of seasonal prevalence from Japanese records, with data from surveys of uncontrolled population groups taken near the end of the study and with data from a village where quinacrine was used routinely to treat proven malaria cases.

Effect on splenomegaly: The over-all picture of the reduction of spleen rates in the study village may be seen in Figures 2, 3 and 4. The total reduction in spleen rates in the study village San Hsing for the entire period of observation was 39 per cent, whereas the reduction of this rate in Liu Chu village (quinacrine—DDT) was 27 per cent. It is to be noted that the spleen rate curve parallels approximately that for parasite rates in Figure 2. Its downward trend continued until the fifth survey, after which it swung up, whereas a rise of the parasite curve is noted after the fourth survey.

The trend of the average enlarged spleen size was progressively downward (Figure 2) except for a slight deviation upward at the fifth survey. Figure 4 shows that the increase in spleen rate which occurred at this time was due principally to an increase in the number of spleens of small size. This finding, when considered with the rising

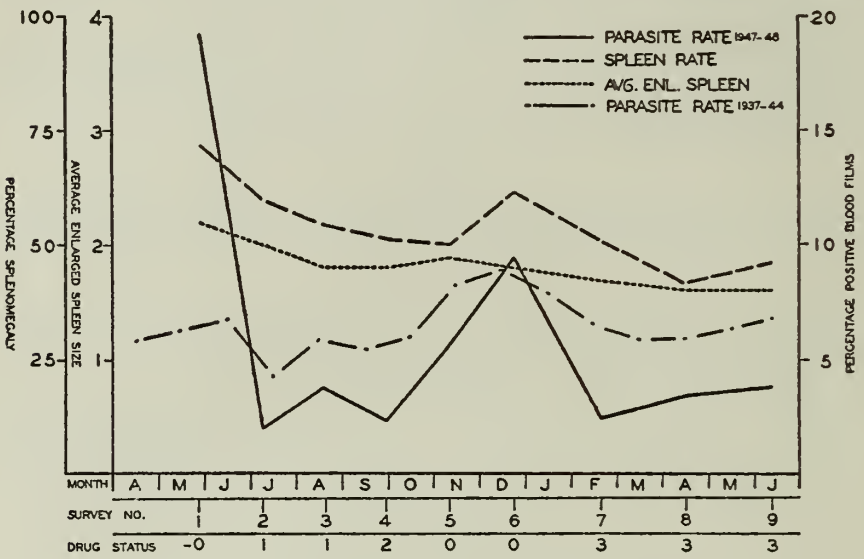

FIG. 2. Parasitemia, Spleen Rates and Size in San Hsing

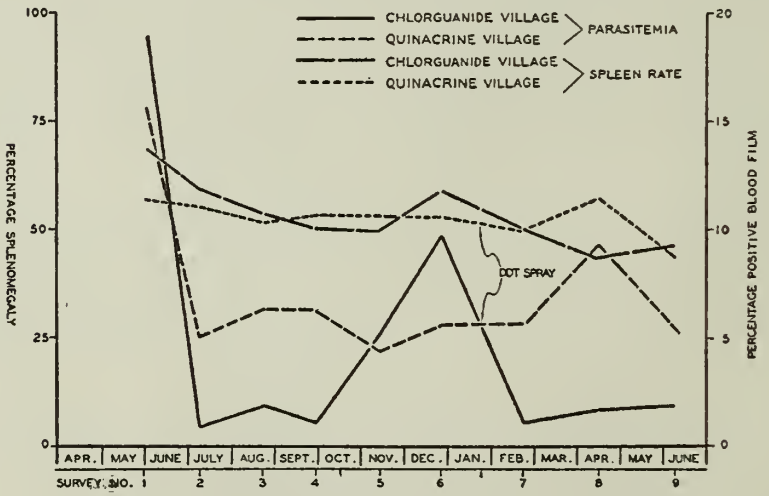

FIG. 3. Comparison of Parasitemia and Spleen Rates in San Hsing and Liu Chu villages.

parasite rate, is suggestive of new infections in the village. The over-all reduction in the average enlarged spleen size was 28 per cent.

Effect on the general level of parasitemia as measured in surveys: For purposes of discussion the study may be conveniently divided into three parts. These correspond

to the initial phase of drug administration (weeks 1 to 18), the intermediate period when chlorguanide was discontinued (weeks 19 to 30) and the final period of drug administration (weeks 31 to 55). Examination of Figure 2 reveals that there was a considerable drop in the rate of parasitemia in the interval between the first two surveys. In fact, the rate for the second survey, 1.9 per cent, was never duplicated though it was approximated in subsequent surveys. The rate of the first survey, 18.2 per cent, may be somewhat lower than that which a survey taken late in April

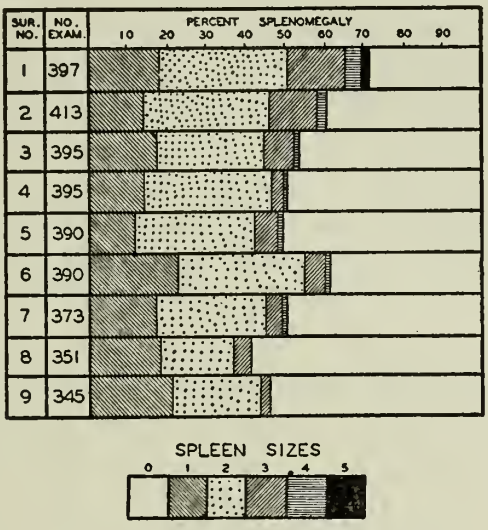

FIG. 4. Splenomegaly in San Hsing Village

TABLE 4

Comparison of Survey Data from Study Villages and from Villages with No Malaria Control

PLACE	DATE	EXAM.	SPLEEN RATE	AVERAGE ENLARGED SPLEEN	PARASITE RATE	PARAS. SPEC. NO.		
						vivax	falc.	mal.
			per cent		*per cent*			
Eighth Survey, Paludrine Study	April 12	315	41.64	1.63	3.42	3	7	2
Ssu Ling, All Ages	April 26	1136	55.58	2.01	13.03	43	44	58
Ru Liao, All Ages	May 9	263	49.80	1.99	14.73	8	13	35
Ka Sa, Primary School	June 12	461	59.20	1.72	14.75	25	10	32
Chu Tien, Primary School	June 26	910	32.97	1.77	11.98	39	21	47
Ninth Survey, Paludrine Study	June 7	345	46.45	1.56	3.77	3	6	4

would have shown. This is suggested by the anophelism curve in Figure 1, but not supported by the composite prevalence curve for 1937–44. There should be a natural decline in prevalence during June and July which may have had some effect on the second survey rate, but certainly was not principally responsible for it. This decline must have been due almost entirely to the administration of chlorguanide in therapeutic and suppressive doses. The rates for the third and fourth survey, while slightly higher than those for the second, do not differ significantly from it.

During the first period of observation it soon became evident that the dosage used was not prophylactic against new infections, or at least against the occurrence of overt parasitemia. These cases were found predominantly in the age groups 3 to 15.[3] Of 23 cases of parasitemia found in the second and third surveys, 20 were in the groups which received from 1.0 to 1.9 mgm./kgm. of chlorguanide per week. The doses were increased until all persons received more than 2.0 mgm./kgm. per week; however, nine cases of parasitemia occurred at the fourth survey, eight of which were in the age groups 3 to 15.

When chlorguanide was discontinued at the fourth survey (19th week) there was a rise in prevalence of parasitemia at the fifth and sixth survey. This would indicate that there was a substantial potential for malaria transmission in spite of the administration of chlorguanide for the preceding 3 months. Fifty-nine cases of parasitemia were found during the fifth and sixth surveys, of which 39 were found in the 0 to 15 age groups and 20 in older age groups. Thus it appears that previous suppression of parasitemia in older age groups, which had received at least 2.0 mgm./kgm. per week throughout the study, had been aided by the influence of immune processes. With the resumption of suppressive therapy at the sixth survey

TABLE 5

Schedule for the Administration of Quinacrine in Liu Chu Village

WEIGHT	DAILY DOSE	TOTAL DOSE (5 DAYS)
Kgm.	*Grams*	*Grams*
9 or less	0.05	0.25
10 to 19	0.10	0.50
20 to 39	0.20	1.00
40 or more	0.30	1.50

(31st week), rates fell to levels comparable to those of the third survey. The preponderance of infections in the younger age groups continued as before: 18 of 34 positive blood films in the last 3 surveys were found in these groups. The ninth survey also revealed that 2 children in the youngest age group (0–2 years), which had received the highest dosage of 3.9 mgm./kgm., were infected.

The curve of prevalence of parasitemia as determined by these 9 surveys is qualitatively quite similar to the 1937–44 curve, a similarity which is increased by the discontinuance of the drug when a seasonal rise was to be expected. There is little doubt that this section of the curve would have leveled off had the drug been continued during the period and that the rates for the eighth and ninth surveys would have been considerably higher if the drug had not been resumed. This conjecture is supported by survey data from several uncontrolled villages (Table 5).

[3] It should be explained that the appearance in tables of "Age in Chinese Years" refers to the system generally employed throughout China to measure age. By this system, a child is considered to be one year old at birth and becomes two years old at the next lunar new year. Thus, a child born on the last day of the old year is considered to be two years old on the next day. To obtain accurate ages the actual birthday must be known and this is often difficult to obtain. Therefore, the Chinese system of denoting ages is employed throughout this study.

Liu Chu village is about 1 kilometer from San Hsing village and had a population of 578 at the time of the first survey. All persons who claimed to be sick and whose blood films contained malaria parasites were given quinacrine according to the schedule in Table 5. The actual taking of the drug was not supervised. Surveys of this village were repeated at the same time as those of San Hsing village and weekly visits were made in order to determine the rate of occurrence of malaria. All proven cases of malaria were treated with quinacrine until January 26, 1948. Then Liu Chu village was used for training personnel in the application of DDT residual spray. One month later DDT spray was reapplied because the first application was not satisfactorily done. Figure 3 shows a comparison of parasite rates for this village and for San Hsing village. While there was reduction of parasite carriers, which can be attributed to treatment with quinacrine and after February to DDT residual spray,

TABLE 6

Numbers of Persons Exhibiting Parasitemia at Surveys, by Age Groups

AGE IN CHINESE YEARS	MEAN WEIGHT	MEAN NO. EXAMINED EACH SURVEY	SURVEY NUMBER									TOTAL 7, 8, 9
			1	2	3	4	5	6	7	8	9	
	Kgm.											
1 & 2	6.4	10	1	0	0	0	1	0	0	0	2	2
3–5	11.8	34	19	4	2	2	3	6	2	2	3	7
6–10	17.2	62	13	2	8	3	5	9	3	1	3	7
11–15	26.9	49	11	0	4	3	3	12	0	2	2	4
16–20	45.3	55	13	1	0	1	7	3	2	2	1	5
21–30	50.1	69	7	0	1	0	1	3	1	4	2	7
31–40	49.6	52	7	0	0	0	1	3	1	1	0	2
41–50	50.0	23	3	1	0	0	1	0	0	0	0	0
50 & Up	45.7	29	0	0	0	0	0	1	0	0	0	0
All Ages		383	74	8	15	9	22	37	9	12	13	34
Per cent Positive			18.2	1.9	3.8	2.3	5.6	9.5	2.4	3.4	3.8	

s

the rates are substantially higher than for San Hsing village during the two period when chlorguanide was administered.

Effect on the occurrence of parasitemia by age groups: The greatest number of cases of parasitemia were found at the first survey, the next greatest number at the sixth survey (Table 6). This could be expected, since no suppressive medication had been given for 13 weeks at the time of the sixth survey. Throughout the study younger age groups had far more infections than the older age groups. In the first survey the age group 0 to 20 furnished 54 per cent of the study population and 74 per cent of the infections found. Using mean values for the study population, the same age group comprised 53 per cent of the total population and 80 per cent of all infections found during the entire study. Concentration of malaria infections in the younger component of a population is, of course, a common finding. This may also indicate that chlorguanide therapy, as used, was less effective in the younger elements of the population studied.

Records of individuals exhibiting parasitemia during the study indicate that all three species of parasites observed followed the distribution by age and time mentioned above. In intersurvey periods blood films were taken only from persons complaining of illness or from persons who had demonstrated parasitemia the week before. Occurrence of parasitemia tended to coincide with survey weeks, notably weeks 1 to 31. This circumstance, in addition to the fact that asymptomatic parasitemia was of common occurrence, indicates that these records underestimate considerably the actual amount of parasitemia in the study population.

The effect of chlorguanide on species parasitemia: 1—*Plasmodium vivax:* In the first survey, *P. vivax* infections represented 50 per cent of all infections found. Of 86 infections with *vivax* found during the study, 37 occurred at the first survey.

This species was most sensitive to chlorguanide therapy. Of 86 cases treated, 72 had not demonstrated recurrence of vivax parasites. Two of the 72 cases could not be followed since they were first found in the 55th and last week of the study. Thus, the recurrence rate was 20 per cent. Of the 14 remaining cases, seven had parasitemia one week after the original finding, but these all cleared up after a second course of treatment. Of the other seven cases, three had one recurrence more than three weeks after the original finding and four had two such recurrences. None had three or more recurrences.

There appeared to be no remarkable persistence of gametocytemia. Gametocytes seemed to be cleared from the blood about as readily as trophozoites. However, no special parasitological studies were made to determine the differential effectiveness of chlorguanide on gametocytes and trophozoites. The term "recurrence" is consistently used instead of the term "relapse," in the discussion of parasitemia, because it is impossible to determine whether the return of parasitemia represents the same or another infection.

Of the 86 *P. vivax* infections found, 20 (22 per cent) occurred while suppressive therapy was being administered: 11 cases in the first period of suppression and nine in the second period. Fifteen of the 20 infections were in persons in the 2–20 age group.

2—*Plasmodium falciparum:* Infections with *P. falciparum* were second in number to *P. vivax* infections at the time of the first survey, 21 cases being found. Of 77 cases found in the entire study, 34 occurred originally in the period when chlorguanide was withheld. There was not much difference in the numbers of cases found in the other three periods. Approximately 80 per cent of falciparum infections were found in the 0–20 age group, about the same proportion as for vivax infections.

Of the 77 infections found 46 showed only rings when the original diagnostic film was examined. These cases may or may not represent new infections. Seventeen were found during the two periods of suppressive treatment and 29 (14 and 15) in the first survey and in the interval period. In the remaining 31 instances, the finding of gametocytes in the films indicate infections of some duration. Nineteen instances of gametocyte carriers in the interval period may represent infections acquired during the first period of suppressive therapy.

Falciparum infections were much more resistant to treatment than vivax infections. Discarding five cases found in the 55th week and 23 cases exhibiting gametocytes only when first found, there was a recurrence rate of approximately 50 per cent.

Without regard to gametocytes, 15 of the 77 cases found had three or more recurrences. Since the term "recurrence" implies disappearance and subsequent return of parasitemia, a better term here would be persistence of parasitemia, for the cases which did not clear up quickly tended to exhibit parasitemia for several successive weeks. These persistent cases nearly always showed gametocytes only, or rings and gametocytes. This is in keeping with Fairley's (1946) finding that chlorguanide is ineffective against *falciparum* gametocytes.

3—*Plasmodium malariae:* Of 43 *P. malariae* infections observed in the study, 20 were found at the first survey and an additional 15 in the first treatment period. These 35 cases represent 80 per cent of all infections found. Only one new case was found in the last 23 weeks, in the 55th and last week of the study. This finding may indicate that chlorguanide had a marked effect on the transmission rate of *malariae* in the village. There was a remarkable concentration of infections in the younger age groups. Thirty of 43 cases were found in persons less than 21 years old.

The response of malariae infections to treatment was, in general, similar to the experience with falciparum infections. The recurrence rate was 46 per cent. However, only eight cases recurred after the second course of treatment. Of these, two recurred three times, one recurred five times and five recurred ten or more times. These five cases were instances of persistent parasitemia, the blood films were positive week after week in spite of treatment.

Remarkable persistence of parasitemia: Two cases of falciparum infection and five cases of malariae infection already noted would appear to warrant brief summaries of their histories.

Case 377: Male, 13 years old, 24 kgm., positive for *P. vivax* in the fourth week, when he first came under observation. The vivax infection cleared up promptly with chlorguanide therapy. He received suppressive doses from the fifth through the 18th weeks, according to the weight schedule. In the interval period his blood films were negative in the 19th and 25th weeks (fourth and fifth surveys) and he made no complaint of illness. At the sixth survey (31st week) his blood film contained large numbers of all forms of *P. malariae*, which persisted for the next four weeks, during which he received four courses of chlorguanide therapy. The blood films were negative from the 35th through the 38th week, during which suppressive doses of chlorguanide were given. The blood films again became positive for *malariae* in the 39th week and remained positive for 17 consecutive weeks. From the 39th through the 48th week he received 0.4 gm. of chlorguanide per week; from the 49th through the 55th week the dose was doubled so that he received 0.8 gm. per week, equivalent to 10 mgm./kgm. per day of treatment. He complained of feeling ill; he had fever only in the 43rd week. After five days of quinacrine treatment (0.2 gm. per day) infection cleared up promptly.

Case 526: Female, four years old, 14 kgm., positive for *P. malariae* in first survey. Responded promptly to therapeutic course of chlorguanide, which was repeated the following week when *P. vivax* appeared in the blood. Chlorguanide was then reduced to 0.11 gm. per week and blood films remained negative for 28 weeks. *P. malariae* appeared in films from 31st to 39th week, with only gametocytes present in the 37th, 38th and 39th weeks. In the 40th week 0.3 gm. of chlorguanide was given and blood

film became negative. Therapeutic course of the drug was again given in the 41st week and suppressive doses for 14 weeks thereafter. Blood film remained negative until 55th week, when trophosoites of *P. malariae* appeared. These disappeared with quinacrine therapy.

Case 277: Female, six years old, 13 kgm., positive for *P. malariae* in 13th week after 12 weeks of suppressive therapy. Therapeutic doses of chlorguanide were given for two consecutive weeks after which films were negative for 10 weeks. The patient received suppressive therapy from the 15th through the 18th weeks. Blood films were again positive for *malariae* in the 25th, 26th and 27th weeks during which she was given therapeutic doses. Suppressive therapy was given regularly from the 28th through the 38th week during which films were negative. Films were positive for the next 10 consecutive weeks and therapy was resumed and given each week at the rate of 10.3 mgm./kgm. per day of treatment. Films were negative from the 49th week through the 54th week. During this period suppressive therapy was given. At the 55th week *malariae* appeared again in the blood which responded to quinacrine. There was no clinical illness at any time during the year.

Case 425: Female, nine years old, 20 kgm., *P. malariae* infection in the first week accompanied by clinical illness, whereupon therapeutic treatment was given. In the second week suppressive doses were administered which were continued throughout the fifth week since blood films were negative during this period. In the sixth and seventh weeks *P. malariae* and *P. falciparum* trophozoites appeared and the patient was quite ill. Following the usual course of chlorguanide therapy in the sixth week, one and a half times this dosage was used in the seventh week; 400 mgm. were given in the first course and 600 mgm. in the second course. The usual course of therapy was repeated in the eighth week, although the blood film was negative. Suppressive treatment was given for the next four weeks during which blood films remained negative. *Malariae* and *falciparum* gametocytes returned in the 13th week without clinical illness. Blood films remained constantly positive for *malariae* through the 22nd week and showed *falciparum* trophozoites in the 20th week. Parasitemia was accompanied by illness in the 16th, 20th and 21st weeks. Chlorguanide was given at 0.4 gm. per week in the 14th and 15th weeks, 0.6 gm. per week in the 16th through 20th weeks and 0.8 gm. in the 21st week. One gram of quinacrine was given in the 22nd week since *falciparum* infection persisted accompanied by illness, whereupon blood films became negative and remained so through the 55th week. Chlorguanide was continued in suppressive doses.

Case 449: Female, nine years old, 17 kgm., *P. malariae* infection accompanied by illness in the 13th week after 12 consecutive weeks of chlorguanide suppressive treatment. Blood films remained positive for *malariae* through the 23rd week, when the infection was terminated by 0.75 gm. of quinacrine. The patient's worsening clinical condition starting in the 15th week prompted the use of quinacrine. During the 13th through 15th weeks 0.2 gm. of chlorguanide per week was given, 0.3 gm. per week from the 16th through the 20th weeks and 0.4 gm. in the 21st and 22nd weeks. In the 31st week blood film was positive for *P. vivax*, which responded promptly to 0.2 gm. of chlorguanide. Thereafter, suppressive chlorguanide was given and blood films remained negative.

Case 450: Male, three years old, 13 kgm., after six weeks of suppressive treatment positive for *P. falciparum* (rings). Therapeutic treatment was given, whereupon rings disappeared. Gametocytes were found in the films during the eighth and ninth weeks. Blood films became negative after therapy and remained so through the 30th week. Suppressive therapy was administered according to schedule. Blood films were again positive for *falciparum* from the 31st through the 33rd weeks and from the 39th through the 47th weeks, after which they were negative. Gametocytes were found in all positive blood films and were accompanied by ring forms in the 32nd, 33rd and 42nd weeks. Therapeutic and suppressive doses were given according to schedule.

Case 338: Female, six years old, 15 kgm., positive for *P. falciparum* the first week of study and remained so for 19 consecutive weeks, during which chlorguanide was given every week. Gametocytes were present in all blood films except the first and ring forms were seen in the blood films for the first, second, sixth, 11th, 12th, 13th and 18th weeks. Chlorguanide was supplemented with pamaquin in the 19th and 20th weeks and blood films became negative in the 20th week. Thereafter chlorguanide alone was used and was given only when the blood film was positive, i.e. during the 22nd and 23rd weeks (*P. falciparum* rings), the 24th week (*P. falciparum* rings and gametocytes), the 39th week (*P. vivax*), the 41st week (*P. falciparum* rings), the 43rd week (*P. falciparum* rings and gametocytes), the 44th week (*P. falciparum* gametocytes) and the 47th week (*P. falciparum* rings). The patient was ill only during the 22nd and 23rd weeks.

The occurrence of malaria illness in relation to parasitemia: Illness was characterized by the presence of fever as demonstrated by a mouth thermometer, with or without general malaise or other complaints. At the beginning of the study approximately nine per cent of persons with parasitemia were ill. This ratio was approximately doubled throughout the first period of chlorguanide administration (Schedules 1 and 2). During the interval period, the ratio was approximately 30 per cent, but fell off to about 4.5 per cent in the last period of chlorguanide administration.

Eighty per cent of all cases of illness in all periods of observation occurred in the age groups 0 to 20 years, following closely the occurrence of overt parasitemia, as would be expected.

Toxic effects: No toxicity attributable to chlorguanide was found during the entire study period.

DISCUSSION

Effectiveness of chlorguanide suppressive treatment: We cannot conclude from our data that our use of chlorguanide resulted in causal prophylactic effects, even against falciparum infections. We did not expect to demonstrate such an effect. In confirming Fairley's (1946) discovery of the prophylactic action of chlorguanide against falciparum infections, Packer (1947) demonstrates that suppressive therapy with chlorguanide should be given at intervals of five days, or less. Packer used doses ranging from 1.25 to 2.0 mgm. per kilogram of body weight (100 mgm. of salt equivalent to 85 mgm. of chlorguanide base per patient). This dosage is roughly comparable to doses we employed. We believe that there may have been fortuitous instances of prophylactic effect against falciparum infections in our study, but have no evidence to support this.

As ordinarily employed, the term "suppression" indicates prevention of the occurrence of clinical manifestations of malaria infections by an agent which inhibits the normal development of malaria parasitism. We had hoped that the doses of chlorguanide employed by us might consistently produce suppression of parasitemia to submicroscopic levels, but this was not the case when suppressive doses or therapeutic doses of chlorguanide were employed.

We have not been able to find many references to work similar to this study; however, roughly comparable reports suggest similar experiences. Lomax (1947) who reported on suppressive use of chlorguanide in Assam villagers found that 100 mgm. per week for adults and 50 mgm. for children "was not a completely reliable suppressive dose." When 100 mgm. was given twice per week better results were obtained, but 200 mgm. once a week did not give much better results than the smaller dose once a week. Viswanathan and Baily (1947) report that weekly chlorguanide doses of 100 mgm. for adults, 50 mgm. for children 5 to 12 years old and 25 mgms. for children two to four years old gave satisfactory, but not entirely effective, results. They recommend twice weekly doses if suppression is sought, but consider that weekly prophylaxis is not practicable.

The schedule of treatment we last employed is probably fairly close to the minimum dosage that can be used with any satisfaction in Formosa. Under this drug regimen five clinical cases developed (four *falciparum*, one *vivax* and one *malariae*) for a rate of 0.25 cases per week. At the same time there were 110 instances of parasitemia in 38 persons. This is suggestive of the persistence of parasitemia in a limited number of persons. We are now of the opinion that, while suppressive doses should be increased for the younger age groups over the doses last used, satisfactory suppression might be obtained with bi-weekly doses at the same level as last given. For administrative and financial reasons, we now hold the view that either diminishing the interval of suppressive doses or increasing the size of the doses would be unfeasible under prevailing Formosan conditions.

Persistence of parasitemia: The five cases of malariae infection and the 2 cases of falciparum infection which developed parasitemia while receiving suppressive therapy and which proved to be refractory to treatment suggest a situation similar to that reported by Bishop and Birkett (1947) and confirmed by Williamson, Bertram and Lourie (1947) and Bishop and Birkett (1948). The latter found that when chicks infected with *Plasmodium gallinaceum* were treated for 30 days with chlorguanide at the rate of 1.25 mgm. per kilogram body weight, the parasites developed great resistance to subsequent treatment with chlorguanide when the amount first used was doubled. Larger initial doses of chlorguanide created a very high degree of resistance to subsequent treatment with chlorguanide; moreover, this factor of resistance was persistent after five mosquito passages of the parasite strain. This resistance did not extend to treatment with quinacrine. Schmidt *et al.* (1949) have shown that a high degree of resistance to chlorguanide can be developed by *Plasmodium cynomolgi* when monkeys infected with this parasite are treated with inadequate doses of the drug. This resistance extended to parasites that had passed through mosquitoes, but resistance to chlorguanide did not make infections insusceptible to treatment with 4-aminoquinolines.

All of the cases we report had received suppressive treatment with chlorguanide at the rate of roughly 2 mgm. per kilogram body weight for varying lengths of time before parasites appeared. All cases subsequently proved to be refractory to treatment with chlorguanide, but all cases cleared up when quinacrine was given. Malariae infections are comparatively difficult to clear up with any sort of treatment, but falciparum infections appear to be rather sensitive to chlorguanide. Therefore, the persistence of the two falciparum infections seems particularly noteworthy.

This finding is somewhat disconcerting with respect to suppressive therapy with chlorguanide in general. It seems possible that doses of chlorguanide sufficient to prevent the occurrence of malaria illness, but insufficient to rid the body of infection, may result in the development of chlorguanide resistant strains.

Administrative aspects of the study: All surveys were under the direction of a physician who was usually assisted by a physician-in-training. The survey team also included one or two technicians for taking blood films and one clerk. Administration of suppressive and therapeutic doses of chlorguanide took about two hours per week at one visit. Although some persons were originally recalcitrant in taking the drug, most villagers were eager to receive it at the outset of the work. As the weeks passed there was less eagerness to take the drug in the absence of illness. However, the study was concluded without much difficulty. This unwillingness to take suppressive therapy is a very real hazard to the use of suppressive therapy as a primary control measure on a large scale. It is our present view that chlorguanide as a suppressive agent should not be used routinely in Formosa as a primary control measure. In the face of a malaria epidemic the drug could be used profitably as a suppressive pending the application of other control measures.

For treatment of clinical malaria, chlorguanide, in our view, is currently the drug of first choice for general use in Formosa. It is the cheapest antimalarial drug on the Far Eastern market today, with the exception of quinacrine. In Hong Kong, quinacrine can be bought at retail for less than the wholesale price of ton lots in the United States, with usual discounts. Quinine sulfate in five grain tablets is currently selling in Hong Kong for about HK$ 120 (US$ 24) per kilogram. This compares with the cost of chlorguanide in Hong Kong of HK$ 270 (US$ 54), in 0.1 gm. tablets; and with the cost of chloroquine (SN 7618) in Manila, in 0.25 gm. tablets, at about three times this price. Our limited experience with chloroquine would indicate that any advantage it may have over chlorguanide on the basis of efficiency is offset by possible toxic effects and would not justify the difference in price.

Chlorguanide today costs about US$ 60 per kilogram delivered in Formosa. On this basis suppressive treatment of the mean study population (383 persons) under Schedule 3 costs US$ 1.71 per week (28.45 gms.) and would have cost US$ 93.88 for study period of 55 weeks. The mean yearly cost per capita on this basis would have been approximately US$ 0.25, or about US$ 1.00 per family of four persons per year. We have demonstrated, however, that adequate suppression was not accomplished by Schedule 3, at least for the younger age groups. If persons less than 16 years of age were given twice as much chlorguanide as the amounts actually administered, while older persons received the regular doses according to schedule, the cost would have been US$ 119 per year and US$ 1.25 per family per year, approxi-

mately. Such costs could not be borne by the average Formosan family, though it is difficult to estimate what each family can afford to pay for suppressive therapy. It appears now that the interval between suppressive doses should be reduced to not more than five days. At this interval, there would be a 27 per cent increase in drug cost over the cost at weekly intervals with the same dose.

It is more difficult to estimate the cost of chlorguanide therapy of clinical malaria in Southern Formosa, for we have no data on the occurrence of clinical malaria in populations not under suppressive therapy or some other control measure. Assuming the need for antimalaria therapy for every member of a family once each year, that the average family is composed of two adults and one child in each age group and that treatment would be given as in Schedule 3, the cost would be about US$ 0.13 per year. Supposing that four times as much chlorguanide is used in the same family per year, since treatment Schedule 3 is not adequate, the cost would be US$ 0.53 per year, approximately half the cost of suppressive therapy as employed by us and possibly within the means of the average Formosan family.

SUMMARY

A Southern Formosan village with a population of 417 was kept under observation for 55 consecutive weeks, during which chlorguanide was administered to the entire. population after proven cases of malaria had been treated with therapeutic doses. Suppressive doses were given for the first 18 weeks of the study, discontinued from the 19th through the 30th weeks, resumed in the 31st week and continued throughout the rest of the study period. All persons weighing 40 or more kilograms (in effect, persons over 15 years old) received 100 mgm. of chlorguanide once each week throughout the study. Younger persons received smaller doses according to weight. The initial dosage schedule proved inadequate and the dosage for children was increased, so that all age groups received suppressive medication at the rate of about 2.0 mgm. per kilogram body weight. An exception was the case of infants, who received, in the last schedule employed, nearly 4.0 mgm. per kilogram.

Persons whose blood films contained malaria parasites and who complained of illness received treatment for three days with chlorguanide. The daily dose was given as a single dose, and averaged from 2.0 to 6.0 mgm., approximately, per kilogram body weight. In the dosage regimen finally employed and used for the longest period (34 weeks) the maximum dose for a single day was 50 mgm. for persons less than six years old, 200 mgm. for persons 6 to 15 years old and 300 mgm. for persons 16 years old or older.

The effectiveness of chlorguanide administration was measured in nine blood and spleen surveys at intervals of six to eight weeks and also by weekly visits to determine the presence of malaria illness in villagers. The initial parasite rate was 18 per cent; of 75 positive blood films in the first survey, 37 contained *P. vivax*, 17 *P. falciparum* and 21 *P. malariae* parasites. In the final survey the parasite rate was 3.8 per cent; of 13 positive blood films three contained *vivax*, six *falciparum* and four *malariae* parasites. During periods when the effect of suppression and therapy could be expected, survey parasite rates were consistently less than four per cent. Persons less than 20 years of age contributed the greater proportion of parasitemia

found and this is believed to be due partly to relative ineffectiveness of the dosages employed in this age group.

The spleen rate dropped from 72 per cent to 46 per cent, a net reduction of 36 per cent. The average enlarged spleen size was reduced from 2.16 to 1.56, a reduction of 28 per cent during the study period.

Vivax infections responded best to therapy, with a recurrence rate of about 20 per cent; recurrence rates for *falciparum* and *malariae* infections were approximately 50 and 46 per cent, respectively.

It is concluded that suppressive doses employed were too small for satisfactory suppression of malaria under Formosan conditions although the maximum doses were probably very near the minimum doses that might be employed. It is tentatively concluded that suppressive therapy with chlorguanide should not be considered as a primary malaria control measure for routine use in Southern Formosa although it might be used to advantage under epidemic conditions. Arguments based upon economic and administrative considerations are presented to support this point of view.

With regard to treatment of malaria cases, chlorguanide appears to be the drug of choice under current conditions in Formosa. It is efficient, relatively cheap and neither toxic manifestations from its use nor staining of the skin were observed throughout the study. Five cases of *malariae* infection and 2 cases of *falciparum* infection proved to be remarkably refractory to treatment with chlorguanide. It is suggested that these cases may represent an acquired tolerance of the parasites to chlorguanide induced by the suppressive regimen employed.

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Dr. Y. S. Huang, a former member of the field staff of the Taiwan Malaria Research Institute, was associated with this study during the first 6 months of observations. The authors desire to acknowledge their appreciation of his participation in field work reported.

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SUMARIO

Una villa del Sur de Formosa con una población de 417 habitantes se mantuvo bajo observación durante 55 semanas consecutivas durante las cuales se administró cloroguanida a toda la población después que los casos comprobados de malaria fueron tratados con dosis terapéuticas. Se administraron dosis supresivas durante las primeras 18 semanas del estudio interrumpiéndose desde la semana 19 hasta la 30, restableciéndose el tratamiento desde la semana 31 durante el resto del período de estudio.—Todas las personas cuyo peso fuera de 40 o más kilogramos (personas mayores de 15 años) recibieron 100 mgms. de cloroguanida una vez por semana durante todo el período de estudio. Las personas más jóvenes recibieron dosis también menores de acuerdo con el peso. La dosificación inicial resultó inadecuada y la dosis para niños hubo de ser aumentada en forma tal que todos los grupos etarios recibieron 2.0 mgm. por kilogramo de peso del cuerpo. Una excepción fué el caso de infantes quienes recibieron, según el último plan empleado, aproximadamente 4.0 mgm. por kilogramo de peso del cuerpo.

Personas con láminas de sangre positivas a parásitos de malaria y quienes se quejaban de enfermedad recibieron tratamiento de tres días con cloroguanida. La dosis diaria fué suministrada en una sola toma y su promedio varió de 2.0 a 6.0 mgm. aproximadamente por kilogramo de peso del cuerpo. En el régimen finalmente empleado, el cual se usó durante el período mayor de tiempo (34 semanas) la dosis máxima por día fué de 50 mgm. para personas menores de 6 años, 200 mgm. para personas entre 6 y 15 años y 300 mgm. para personas de 16 y más años de edad.

La efectivadad de la administración de la cloroguanida fué medida por medio de 9 índices parasitarios e igual número de índices esplénicos efectuados a intervalos 6 a 8 semanas y también por visitas semanales para determinar la presencia de la enfermedad entre los habitantes. El índice parasitario inicial dió 18 por ciento; de 75 láminas positivas en la primera encuesta, 37 lo fueron a *P. vivax*, 17 a *P. falciparum* y 21 a *P. malariae*. En la última encuesta el índice fué de 3.8 por ciento y de 13 láminas positivas 3 lo fueron a *vivax*, 6 a *falciparum* y 4 a *malariae*. Durante los períodos en que podrían esperarse efectos supersivos y terapéuticos las encuestas indicaron constantemente índices parasitarios menores que 4 por ciento. Las personas menores de 20 años produjeron la mayor proporción de parasitemia lo que podría atribuirse parcialmente a la relativa inefectividad de las dosis empleadas en este grupo etario.

El índice esplénico bajó de 72 a 46 por ciento, lo que significa una reducción neta de 36 por ciento. La esplenomegalia media fué reducida de 2.16 a 1.56 o sea una reducción de 28 por ciento durante el período del estudio.

Las infecciones a *vivax* fueron las que mejor respondieron a la terapéutica con aproximadamente 20 por ciento de recaídas. Las recaídas para *falciparum* y *malaria* fueron del 50 y 46 por ciento, respectivamente.

Se concluye que las dosis supresivas empleadas fueron muy pequeñas para lograr una supresión satisfactoria de malaria bajo las condiciones en Formosa aunque las dosis máximas usadas estuvieron muy cerca de las dosis mínimas que podrían usarse. Se concluye tentativamente que la terapéutica supresiva con cloroguanida no debe considerarse como una medida primordial de rutina para control de malaria en Formosa del Sur, aunque pudiera usarse con ventaja bajo condiciones epidémicas. Se presentan argumentos basados en consideraciones administrativas y económicas para respaldar este punto de vista.

En lo que respecta a tratamiento de casos de malaria, la cloroguanida parece ser la droga a elegir bajo las condiciones normales en Formosa. Es eficiente, relativamente barata y no se observaron durante el tiempo que duró el estudio manifestaciones tóxicas ni coloración de la piel que pudiera atribuirse a las misma. Cinco casos de infección a *malariae* y 2 a *falciparum* se mostraron notablemente refractarios al tratamiento con cloroguanida. Esto sugiere que estos casos pudieran representar una tolerancia adquirida de los parásitos provocada por el régimen supresivo empleado.

RESULTS ON 449 CASES OF NATURALLY ACQUIRED MALARIA TREATED WITH CHLOROQUINE[1]

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Although considerable work had been done proving the efficacy of chloroquine in the treatment of malaria, it was the purpose of this investigation to determine the optimal dosage of this drug in the treatment of naturally acquired *falciparum* and *vivax* malaria in a highly immune adult native population. It was also hoped that we might carefully study the nature and severity of symptoms attributable to drug intolerance. Additional purposes of the project were to study the possibility of gametocidal activity in *falciparum* malaria and, finally, to determine the value and intolerance of the drug over a wide age variable.

WHERE STUDIES WERE MADE

Although the United Fruit Company has 14 completely equipped hospitals throughout the tropics, it was necessary to concentrate the work as much as possible. For this reason, three hospitals were chosen which would give us the best distribution of falciparum and vivax malaria and where facilities for clinical research were maximal. With these points in mind, one hospital in Panama, Costa Rica, and Honduras was selected. Previous efforts to place the project over a more extensive area failed to bring about the desired results as the pressure of work in many of these hospitals was too great to enter into a detailed clinical research project.

ROUTINE EMPLOYED

The hospitals ultimately collaborating in this investigation were provided with uniform record charts covering the personal history of the patient. They were instructed to take temperatures every four hours and blood smears every 12 hours until such time that normal temperatures were sustained for 24 hours and no parasites were found in thick smear preparations. Before treatment was initiated, thin smears were also requested in order that an arbitrary index of parasitemia might be established, and the possibility of treating mixed infections eliminated. The physicians were requested to routinely make careful observation on all patients undergoing treatment and to record any manifestations of drug intolerance. In many instances

[1] In presenting this analysis of cases of naturally acquired malaria treated with chloroquine, it becomes necessary that the conditions under which this project were carried out be clearly understood.

The actual treatment of all cases was carried out by physicians of the United Fruit Company, and all laboratory work was performed by technicians of that company. Therefore, to the medical and technical groups of the United Fruit Company I am deeply indebted for their efforts which made this report possible. The Chloroquine used in this study was Aralen Diphosphate furnished by the Winthrop-Stearns, Inc.

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the hospitals would be working simultaneously on the same dose schedule. As the investigation continued, each hospital was assigned a different dose schedule.

When treating children, regardless of the dose schedule, Young's rule was employed.

The 449 cases included in this report represent, after close scrutiny of the case histories, those which contain complete and pertinent data. However, in presenting this analysis I must rely entirely upon the data obtained by others as none of these treatments was carried out under my direct supervision. In any case where any doubt existed in the case history, it was eliminated from this report.

Due to conditions beyond our control it was impossible to determine any plasma levels throughout this entire study.

At the beginning, the generally accepted dose schedule was employed, i.e., an initial dose of 1.0 gram of chloroquine followed 8 hours later by an additional 0.5 gram. A single dose of 0.5 gram was then given on each of two consecutive days (Method I). As results were accumulated, the total dose of the base and the time of administration was gradually reduced. The dosage and time factor were reduced further in an effort to establish a point at which the drug showed no therapeutic effect. By reference to Tables 1 and 2, it will be noted that this point was never reached as single small doses of the drug generally appeared almost as satisfactory in this adult immune group as large doses extended over a three-day period.

RESULTS OBTAINED

(Falciparum *Malaria*)

Reference to Table I shows 247 cases of naturally acquired *falciparum* malaria treated with chloroquine. The dosage ranged from 2.5 grams of chloroquine (1.56 grams of base) administered over a period of 3 days to 0.125 gram of chloroquine (0.08 gram of base) administered in a single dose. Regardless of the method or dosage employed there seems to be relatively little difference in the time for return to maintained normal temperature and the parasite clearance time. There are two significant observations in this analysis. First, that Method 10, which consisted of an initial dose of 0.6 gram of chloroquine followed 8 hours later by another dose of 0.6 gram (total dose of base of 0.75 gram) appears to be an effective method for administration of this drug. By this method, 82 per cent of the cases were cleared of parasites within 48 hours, and 94 per cent of those with fever had returned to maintained normal temperature within 48 hours. Of the 57 cases treated by this method, only one evidenced any symptoms of drug intolerance. In this case mild abdominal pain was noted. It is extremely difficult to state whether or not that such a complaint was due to drug administration or to one or another condition so prevalent among the native population of these countries.

In four cases treated by this method both sexual and asexual forms of parasites were observed. Of these, one was free of gametocytes 36 hours following administration of the initial dose. In the other three, the gametocytes persisted for more than 96 hours, at which time plasmoquin was given.

Another point of interest in this study was the fact that doses lower than 1.5

TABLE 1

Falciparum malaria (247 cases)

	METHOD OF ADMINISTRATION OF CHLOROQUINE	TOTAL BASE DOSE: GMS.	TOTAL NUMBER OF CASES TREATED		DEGREE OF PARASITEMIA*				FORMS OBSERVED		AGE DISTRIBUTION				RETURN TO MAINTAINED NORMAL TEMPERATURE						PARASITE CLEARANCE TIME					SYMPTOMS OF DRUG INTOLERANCE		REMARKS
			Male	Female	+	++	+++	++++	Asexual	Sexual	Under 1 year	1–10 years	11–20 years	Over 20 years	Afebrile	Less than 12 hrs.	12–24 hours	24–48 hours	48–72 hours	72–96 hours	Within 24 hrs.	24–48 hours	48–72 hours	72–96 hours	Over 96 hrs.	Mild	Severe	
1	1.0 Gm. Initial dose 0.5 Gm. 8 Hours later 0.5 Gm. Daily for 2 days	1.56	24	6	?	?	?	?	30	0	0	1	9	20	10	3	8	8	0	1	5	10	11	4	0	1	0	Headache (1 case) Degree of parasitemia not stated
2	1.5 Gm. Initial dose 1.0 Gm. Following day	1.56	6	1	0	0	4	3	7	0	0	1	1	5	1	0	1	4	0	1	3	4	0	0	0	3	0	Vomiting (1 case) Itching (2 cases)
3	1.0 Gm. Initial dose 0.3 Gm. 8 Hours later 0.3 Gm. Daily for 2 days	1.19	25	1	18	5	3	0	24	6	0	0	3	23	7	4	5	8	1	1	8	11	1	3	3	2	0	Itching (2 cases)
4	0.6 Gm. Initial dose 0.3 Gm. 4 Hours later 0.3 Gm. Daily for 3 days	1.12	2	2	2	1	1	0	4	0	1	0	1	2	1	0	1	2	0	0	2	2	0	0	0	0	0	
5	0.6 Gm. Initial dose 0.6 Gm. Daily for 2 days	1.12	3	0	0	1	0	2	3	0	?	?	?	?	1	2	0	0	0	0	2	1	0	0	0	0	0	No ages recorded
6	0.3 Gm. Initial dose 0.3 Gm. 4 Hours later 0.3 Gm. Daily for 3 days	0.94	14	6	?	?	?	?	17	3	0	1	5	14	0	1	9	7	3	0	0	13	0	4	3	2	0 0	Headache (1 case) Headache & dizziness (1 case)
7	0.5 Gm. Initial dose 0.5 Gm. 2 doses**	0.94	6	5	5	2	1	3	11	2	?	?	?	?	3	0	5	2	0	1	0	4	4	3	0	1	0	No ages recorded
8	0.6 Gm. Initial dose 0.3 Gm. 3 doses**	0.94	21	2	6	11	1	5	9	1	0	1	5	17	2	1	9	11	0	0	9	11	3	0	0	0	0	In 13 cases: parasite form undifferentiated
9	0.7 Gm. Initial dose 0.5 Gm. Following day	0.75	4	0	2	2	0	0	4	0	0	1	2	1	0	2	1	1	0	0	1	3	0	0	0	0	0	
10	0.6 Gm. Initial dose 0.6 Gm. 8 hours later	0.75	47	10	23	2	0	32	57	4	0	2	11	44	10	6	22	16	2	1	7	40	7	0	0	1	0	[1]
11	0.5 Gm. Initial dose 0.5 Gm. 8 hours later	0.62	5	1	1	0	1	4	6	1	0	0	3	3	1	1	2	1	1	0	0	5	0	1	0	0	0	[2]
12	1.0 Gm. Initial dose‡	0.62	3	2	1	1	0	3	5	1	0	0	3	2	1	0	0	2	2	0	0	4	1	0	0	0	0	[3]
13	0.75 Gm. Initial dose‡	0.47	6	7	5	1	1	6	13	1	0	1	4	8	0	2	5	3	3	0	0	11	1	1	0	0	0	[4]
14	0.5 Gm. Initial dose‡	0.31	6	9	7	0	0	8	14	1	0	2	2	11	2	1	5	6	1	0	4	8	2	1	0	0	0	[5]
15	0.25 Gm. Initial dose‡	0.16	12	5	8	0	1	8	17	0	0	0	8	9	2	2	6	5	2	0	3	8	3	2	1	0	0	
16	0.125 Gm. Initial dose‡	0.08	5	1	2	0	0	4	6	0	0	0	2	4	3	0	0	1	1	1	1	4	1	0	0	0	0	
Totals†			189	58											44	25	79	77	16	6	45	139	34	19	7	10	0	

* Degree of parasitemia: 0–1 parasites in 10 fields (thin smear) +: 2–5 parasites in 10 fields (thin smear) ++: 6–10 parasites in 10 fields (thin smear) +++: Over 10 parasites in 10 fields (thin smear) ++++. † 3 cases treated by method 10 failed to clear. ‡ No further treatment. ** 8 hrs. apart.

[1] In 1 case the gametocytes cleared in 36 hrs. In 3 cases the gametocytes failed to clear. Mild abdominal pain (1 case).

[2] Rings cleared, 36 hrs. Gams. cleared. 108 hrs.

[3] Rings cleared, in 36 hrs. Gams. cleared, 60 hrs.

[4] Rings & gams. cleared, 36 hrs.

[5] Gam. cleared, 12 hrs.

TABLE 2

Vivax malaria (202 cases)

	METHOD OF ADMINISTRATION OF CHLOROQUINE	TOTAL BASE DOSE: GMS.	TOTAL NUMBER OF CASES TREATED		DEGREE OF PARASITEMIA*				FORMS OBSERVED		AGE DISTRIBUTION				RETURN TO MAINTAINED NORMAL TEMPERATURE						PARASITE CLEARANCE TIME					SYMPTOMS OF DRUG INTOLERANCE		REMARKS
			Male	Female	+	++	+++	++++	Asexual	Sexual	Under 1 Year	1–10 Years	11–20 Years	Over 20 years	Afebrile	Less than 12 hrs	12–24 hours	24–48 hours	48–72 hours	72–96 hours	Within 24 hrs	24–48 hours	48–72 hours	72–96 hours	Over 96 hours	Mild	Severe	
1	1.0 Gm. Initial dose 0.5 Gm. 8 Hours later 0.5 Gm. Daily for 2 days	1.56	29	5	?	?	?	?	34	1	0	0	15	19	3	8	10	7	2	4	11	18	1	3	1	2	0	Headache (1 case) Itching (1 case)
2	1.5 Gm. Initial dose 1.0 Gm. Following day	1.56	3	1	1	0	2	1	4	0	0	0	4	0	0	0	0	4	0	0	1	3	0	0	0	1	0	Slight abdominal pain (1 case)
3	1.0 Gm. Initial dose 0.3 Gm. 8 Hours later 0.3 Gm. Daily for 2 days	1.19	14	2	10	3	1	2	16	13	0	0	5	11	4	1	6	5	0	0	8	6	1	1	0	1	0	Headache (1 case)
4	0.6 Gm. Initial dose 0.3 Gm. 4 Hours later 0.3 Gm. Daily for 3 days	1.12	4	2	4	2	0	0	6	3	0	2	0	4	3	1	0	2	0	0	2	2	0	1	1	0	0	
5	0.6 Gm. Initial dose 0.6 Gm. Daily for 2 days	1.12	4	2	1	1	1	3	5	6	?	?	?	?	1	1	4	0	0	0	5	1	0	0	0	0	0	No ages recorded
6	0.3 Gm. Initial dose 0.3 Gm. 4 Hours later 0.3 Gm. Daily for 3 days	0.94	8	7	?	?	?	?	14	2	1	3	4	7	0	1	7	5	1	1	4	6	3	2	0	0	0	In 1 case, no relapse in 3 months.
7	0.5 Gm. Initial dose 0.5 Gm. 2 doses**	0.94	6	5	7	1	1	2	8	11	?	?	?	?	1	3	2	4	1	0	5	6	0	0	0	0	0	No ages recorded
8	0.6 Gm. Initial dose 0.3 Gm. 3 doses**	0.94	4	1	0	5	0	0	5	1	0	1	1	3	2	1	0	2	0	0	5	0	0	0	0	0	0	
9	0.7 Gm. Initial dose 0.5 Gm. Following day	0.75	5	4	5	4	0	0	9	1	0	0	1	8	2	2	4	1	0	0	8	1	0	0	0	0	0	
10	0.6 Gm. Initial dose 0.6 Gm. 8 Hours later	0.75	43	11	21	3	1	29	29	48	0	3	20	26	9	2	21	21	1	0	22	30	2	0	0	1	0	Abdominal pain (1 case) In 5 cases, no ages recorded
11	0.5 Gm. Initial dose 0.5 Gm. 8 Hours later	0.62	5	1	4	0	0	2	3	5	0	0	3	3	0	1	3	2	0	0	3	2	0	1	0	0	0	
12	1.0 Gm. Initial dose‡	0.62	2	0	0	0	1	1	2	1	0	0	0	2	1	0	0	1	0	0	0	1	1	0	0	0	0	
13	0.75 Gm. Initial dose‡	0.47	10	2	4	0	2	6	5	12	0	1	5	6	2	2	5	3	0	0	5	7	0	0	0	0	0	
14	0.5 Gm. Initial dose‡	0.31	2	0	1	0	0	1	1	2	0	0	0	2	0	0	0	2	0	0	1	1	0	0	0	0	0	
15	0.25 Gm. Initial dose‡	0.16	6	1	3	0	0	4	5	5	0	0	4	3	0	0	0	6	1	0	4	1	1	1	0	0	0	
16	0.125 Gm. Initial dose‡	0.08	11	2	6	0	1	6	8	11	0	0	3	10	3	1	2	1	2	3	0	1	8	1	3	0	0	§
Totals†			156	46											31	24	64	66	8	8	84	86	17	10	5	5	0	

* Degree of parasitemia: 0–1 parasites in 10 fields (thin smear) +: 2–5 parasites in 10 fields (thin smear) ++: 6–10 parasites in 10 fields (thin smear) +++: Over 10 parasites in 10 fields (thin smear) ++++. † 1 case required 7 days for return to maintained normal temperature. ‡ No further treatment. ** 8 hrs. apart. § Return to normal temperature, 7 days (1 case).

gram of chloroquine demonstrated no untoward symptoms attributable to the drug. The only exception to this statement is that just referred to. In the 247 cases of *falciparum* malaria treated with chloroquine only ten (2.23 per cent) complained of any symptoms which might be attributed to the drug. All of these were mild and might or might not be considered as true toxic manifestations. An analysis of these reactions is shown in Table 1.

The apparent gametocidal action of small doses of this drug in the treatment of *falciparum* malaria appears coincidental. However, it might be suggested that this property of the drug in small doses might be investigated more completely.

With very few exceptions the majority of cases of *falciparum* malaria returned to a maintained normal temperature between 12 and 48 hours following the initial dose regardless of the method employed. The great majority of this series showed a parasite clearance time within the first 48 hours from the starting of the treatment. This finding was rather consistent irrespective of the method employed (see Table 1).

(Vivax *Malaria*)

The observations with *vivax* malaria were very similar to those found in the treatment of *falciparum* malaria. Here again Method 10 seemed to be the most effective dose schedule employed. In this series of 202 cases only five (2.48 per cent) showed any evidence of drug intolerance of which four occurred at the higher dose levels. The time for return to maintained normal temperature and the parasite clearance time practically parallels the observations reported in the *falciparum* series (see Table 2). By reference to this table it will be noted that Method 16 in which 0.125 gram of chloroquine was administered, showed a tendency to approach the dosage at which no therapeutic action of the drug would become manifested.

SUMMARY

A total of 449 cases of naturally acquired malaria have been studied. Of this number 247 were *falciparum* infections and 202 were *vivax* infections.

Sixteen dose schedules have been tried (see Tables 1 and 2). The total dose of chloroquine varied from 2.5 grams to 0.125 gram. In 57 cases of *falciparum* malaria and 54 cases of *vivax* infection, Method 10, which consisted of administration of 0.6 gram of chloroquine followed eight hours later by similar dose was an effective means of therapy in both groups. This dosage is one-half that recommended by the Board for Coordination of Malarial Studies, (Loeb 1946) Most et al., (1946) and Earle and Berliner. (1948) Even more important than the reduction in drug dosage is the reduced time necessary to terminate an acute attack of the infection.

Of the total of 449 treated only 15 (3.34 per cent) showed any indication of drug intolerance, and in no case below a dosage of 1.2 grams of the chloroquine were any untoward symptoms observed.

We have found that this drug is effective and non-toxic in young children as well as in adults. Our youngest case treated was 21 days old. In the entire series in which the age was recorded, there were 22 (4.9 per cent) under 10 years of age.

Due to existing circumstances it was impossible to run plasma levels and to determine the effect of chloroquine on relapses of *vivax* malaria.

For suppression, we have found 0.25 gram of chloroquine administered once a week to be entirely effective.

ACKNOWLEDGMENTS

To Dr. Edward I. Salisbury, Medical Director of the United Fruit Company, my deep appreciation for all of the many phases of this study in which he rendered so much assistance.

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SUMARIO

Se presenta el estudio de 449 casos de malaria natural. Entre los mismos 247 fueron de *falciparum* y 202 de *vivax*.

Se probaron 16 planes de tratamiento diferentes (Ver Tablas 1 y 2). La dosis total de cloroquina varió de 2.5 gms. a 0.125 gramos. Para tratar 57 casos de *falciparum* y 54 de *vivax* se siguió el método 10, el cual consiste en la administración de 0.6 gramos de cloroquina seguidos de la misma dosis a las ocho horas. En ambos grupos esta dosis resultó un medio terapéutico efectivo. Esta dosis es la mitad de la recomendada por la Junta de Coordinación de Estudios de Malaria, (Loeb 1946) Most en otra parte (1946) y Earle y Berliner (1948). Aún más importante que la reducción en la dosis de la droga es la reducción del tiempo necesario para terminar con un ataque agudo de la infección.

Del total de los 449 casos solamente 15 (3.34 por ciento) mostraron algún síntoma de intolerancia. En ningún caso de los sometidos a dosis inferiores a 1.2 gramos de cloroquina se observaron síntomas refractarios.

Se observó que la droga fué efectiva y no tóxica tanto en adultos como en niños menores. El caso más joven tratado fué un recien nacido de 21 días. Entre todas las edades registradas durante el estudio hubo 22 (4.9 por ciento) menores de 10 años.

Debido a las circunstancias reinantes no fué posible hacer pruebas de niveles del plasma y determinar el efecto de la cloroquina en las recaídas de infecciones a *vivax*.

Se encontró que para tratamiento supresivo una dosis de 0.25 gramos de cloroquina una vez semanal fué completamente efectiva.

STUDIES IN HUMAN MALARIA

XXII. Prolonged Suppression of Chesson Strain *VIVAX* Malaria By the Weekly Administration of Chlorguanide or Chloroquine

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The study reported in this paper was carried out in prisoner-volunteers at the Federal Correctional Institution, Seagoville, Texas, in an attempt to answer the following questions: (1) Would weekly doses of chloroquine[2] and weekly doses of chlorguanide[3] suppress sporozoite-induced infections caused by the Chesson strain of *Plasmodium vivax*? (2) If effectively suppressed, would the infections outlast six or twelve months of drug administration? (3) If malaria appeared after the period of suppression, would the number and spacing of attacks differ from those in individuals who had not had suppressive drug?

MATERIAL AND METHODS

Subjects: The 27 white male volunteers ranged in age from 23 to 43 years. Two subjects had had luetic infections which had been treated prior to these studies.

Inoculation: The Chesson strain of *P. vivax* came originally from New Guinea (Ehrman *et al.*, 1945) and produces infections which exhibit frequent relapses without long-term latency (Coatney *et al.*, 1949; Craige *et al.*, 1947). Infections were transmitted by insectary-reared *Anopheles quadrimaculatus*, each volunteer being bitten by 10 infected mosquitoes. Infections in the mosquitoes were proved by examination of the salivary glands after the final feeding, the number of sporozoites per pair of glands being graded on a scale of 0 to 4+. By means of interrupted feedings (Coatney *et al.*, 1948), treated and control patients were bitten by the same mosquitoes.

Drugs: Chloroquine was given as the diphosphate, in tablets containing 0.3 gram of chloroquine *base*. Chlorguanide was given as the hydrochloride, in capsules containing 0.15 gram of chlorguanide *base*.

Observations for malaria: With a few exceptions, the observations of the volunteers were similar to those employed by Coatney *et al.* (1948) in studies carried out at Atlanta. Thick blood smears were examined at least twice weekly; at times they were made and examined daily. All the men who were receiving suppressive medication had oral temperature readings made morning and evening from the 10th through the 62nd post-inoculation day. Upon the appearance of patent parasitemia or symp-

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[2] SN 7618 or 7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline.

[3] N_1-(p-chlorophenyl)-N_5-isopropyl biguanide, also known as paludrine, proguanil.

toms, volunteers were hospitalized and placed on a routine of rectal temperature readings, made every four hours. Treatment of acute attacks of malaria was begun at noon of the 3rd, 4th or 5th day of continuous patent parasitemia. Treatment was begun earlier than the 5th day only in those cases where temperature of 102° F. had occurred.

Chemical determinations: Estimations of chloroquine in plasma were made by the photofluorometric method of Brodie *et al.* (1947), in samples drawn as follows: (a) immediately before each of the first 12 suppressive doses; (b) six hours after each suppressive dose; (c) daily for 1 week following the 3rd and the 27th dose; and (d) every other day after the 53rd suppressive dose as long as measurable concentrations were present.

Concentrations of chlorguanide in plasma were estimated by the method of Spinks and Tottey (1946), adapted for measurement of concentrations as low as 10 micrograms per liter. Samples of blood were collected as follows: (a) 3 hours after each dose; (b) daily after the 3rd, 4th, 27th and 53rd doses, as long as measurable concentrations persisted.

Studies of complement fixation: Blood was drawn one to three times each week for study of complement fixing antibodies, using a *P. knowlesi* antigen. These tests were carried out by the Army Medical Research and Graduate School; the results will be incorporated in a separate report.

EXPERIMENTS AND RESULTS

The 27 volunteers included in the experiment were all bitten by heavily-infected mosquitoes from the same lots. These mosquitoes fed on subjects S-15 through S-26 on 1 September 1946 and on subjects S-27 through S-41 on 2 September 1946.

Nine volunteers (S-15 through S-18 and S-27 through S-31) were given chloroquine 0.3 gram (*base*) once weekly. The schedule was such that the first 4 subjects began receiving the drug on the 3rd day before the day of exposure, while the last five subjects began receiving the drug on the 7th day before the day of exposure.

Nine volunteers (S-19 through S-22 and S-32 through S-36) were given 0.3 gram of chlorguanide (*base*) once weekly, the schedules of administration being exactly as those for chloroquine.

The nine control subjects (S-23 through S-26 and S-37 through S-41) were given no protective drug. They developed patent malaria 10 to 12 days after exposure and were then treated with various drug regimens. Two of the controls (S-24 and S-41), given quinine sulfate for all attacks, are of particular interest as they provide information as to the relapse pattern of the control infection with non-curative therapy.

No patent parasitemia occurred in any of the volunteers who were receiving protective medication. The only symptoms noted were in three of the five individuals who were given chloroquine beginning seven days before exposure. These three men (S-27, S-29 and S-31) had oral temperatures above 100° F. 10 and 11 days after inoculation, and one had chills with temperature above 102° F. on the 10th and 11th days. Plasma levels of chlorquine, in blood drawn after the onset of fever, were 18, 11 and 15 micrograms per liter, respectively. Symptoms cleared before the next dose

of drug became due (on day 14), and the three patients showed no further symptoms until after discontinuance of medication.

TABLE 1

The effect of weekly doses of chloroquine or chlorguanide upon sporozoite-induced Chesson strain vivax malaria

DRUG REGIMEN	VOL. NO.	SET NO.*	INFECTIVE INOCULUM DAY 0 (TOTAL† PLUSES)	DRUG STARTED (DAYS BEFORE BITES)	DRUG STOPPED (DAYS AFTER BITES)	DAYS FROM LAST DOSAGE TO FIRST POSITIVE BLOOD SMEAR	PREPATENT PERIOD (DAYS AFTER BITES)	INCUBATION PERIOD (DAYS AFTER BITES)
Chloroquine, 0.3 gram base weekly for 26 weeks	S-15	1	40+	3	179	51	230	232
	S-17	3	35+	3	179	44	223	226
	S-27	5	40+	7	175	47	222	10‡
	S-29	7	35+	7	175	45	220	11§
	S-31	9	40+	7	175	57	232	236‖
Chloroquine, 0.3 gram base weekly for 52 weeks	S-16	2	36+	3	361	62	423	424
	S-18	4	40+	3	361	70	431	432
	S-28	6	38+	7	357	58	414	415
	S-30	8	38+	7	357	73	430	431
Chlorguanide, 0.3 gram base weekly for 26 weeks	S-19	1	40+	3	179	24	203	203
	S-21	3	35+	3	179	19	198	199
	S-32	5	40+	7	175	22	197	197
	S-34	7	35+	7	175	24	199	200
	S-36	9	40+	7	175	24	199	199
Chlorguanide, 0.3 gram base weekly for 52 weeks	S-20	2	36+	3	361	18	379	380
	S-22	4	40+	3	361	19	380	380
	S-33	6	38+	7	357	19	376	375
	S-35	8	38+	7	357	23	380	379
Controls	S-23	1	40+	—	—	—	11	11
	S-24	2	38+	—	—	—	11	13
	S-25	3	35+	—	—	—	11	13
	S-26	4	37+	—	—	—	12	12
	S-37	5	40+	—	—	—	11	13
	S-38	6	38+	—	—	—	10	10
	S-39	7	37+	—	—	—	11	11
	S-40	8	38+	—	—	—	10	11
	S-41	9	40+	—	—	—	10	10

* Volunteers with the same set numbers were bitten by the same mosquitoes.

† Sporozoite densities in the 10 infected mosquitoes which bit each subject were graded on a scale from 1+ to 4+; an approximation of the inoculum for each man was made by adding the pluses.

‡ Temperature 102.2 to 103.2° on days 10 and 11.

§ Temperature of 101.6° on day 11.

‖ Temperature of 100.4° on day 10.

At the end of six months, suppressive medication was discontinued in five subjects who were receiving chloroquine and in five who were receiving chlorguanide. At the

end of 12 months, suppressive drug was discontinued in the remaining subjects. As can be seen in table 1, all developed acute malaria. The time intervals between the discontinuance of suppression and the appearance of parasitemia varied between 44 and 73 days (mean 56.3 days) for the men who had received chloroquine and 18 and 24 days (mean 21.3 days) for those who had received chlorguanide.

Relapse patterns after discontinuance of suppression: The delayed primary attacks and the subsequent relapses which followed the discontinuance of suppression were terminated with courses of quinine sulfate (2.0 grams of base per day for 14 days)

TABLE 2

Latent intervals following treatment with quinine sulfate, 2 grams per day for 14 days, given during acute attacks of Chesson strain vivax malaria in controls and in subjects in whom malaria had been suppressed for 6 months or 12 months

VOL. NO.	DAYS FROM END OF QUININE TREATMENT TO RELAPSE FOR ATTACKS TREATED DURING:		
	First 6 months	Second 6 months	Third 6 months
S- 7	7 10 10 11 14 16	16 30 103	20 >102*
S-11	6 7 8 8 10 13 15	16 18 29 56	48 >74
S-24	6 8 11 12 13 16	34 49 62	100
S-41	8 8 10 10 11 15 19	24 32 36 25	44 >62
S-15		11 15 10 >211	
S-27		10 9 12 12 12 39	>106
S-19	suppression	8 19 10 31 19	>152
S-21		9 12 22 14 20 39	28 40 >0
S-36		6 9 16 54 >176	
S-16			>108
S-18			14 16 >35
S-28			11 >85
S-30	suppression	suppression	15 >67
S-20			9 15 >94
S-22			15 13 >89
S-33			19 8 9 >66
S-35			6 15 9 37>15

* Indicates no relapse during indicated period of observation after last treatment.

in all volunteers except Nos. S-17, S-29, S-31, S-32 and S-34. The latter men received standard courses of chloroquine (1.5 grams of base in 4 days). Responses to both drugs were prompt.

The pattern of attacks after suppression has been compared with that in the controls (table 2 and figure 1). For the purpose of this comparison, in addition to controls S-24 and S-41, controls S-7 and S-11 from a preceding experiment were included; they had been given a comparable sporozoite inoculation 40 days earlier. These four men, who had no protective drug but were treated with quinine during each acute attack, displayed 10 to 13 attacks of malaria per man during 18 months of observation. Intervals from the end of treatment to reappearance of parasites became progressively longer with successive attacks. Thus the primary attacks were followed by relapse in six to eight days; the first attacks treated after 6 months

were followed by relapse in 16 to 34 days, while the first attacks treated after 12 months were followed by relapse in 20 to 100 days.

In the individuals maintained on suppressive medication for 6 or 12 months and then given quinine for acute attacks, relapses at first occurred rather promptly, but the intervals rapidly widened. The attacks of malaria for each man are diagrammed in figure 1. While it was unfortunate that commitments to the volunteers made it impossible to maintain blood-smear observations beyond 18 months, all of the men had opportunities to report relapses beyond the required observation period. The

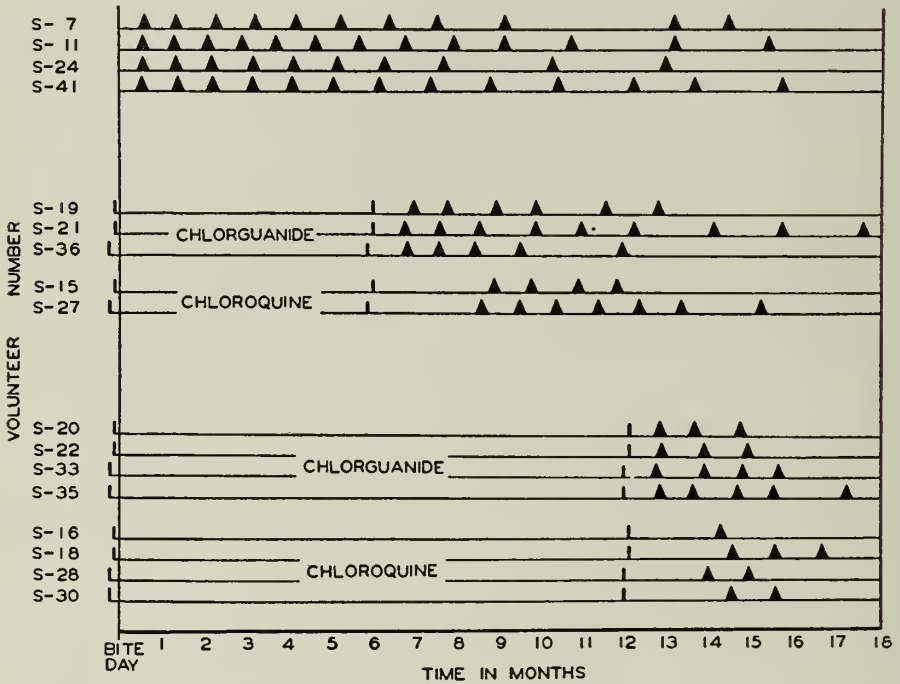

FIG. 1. Diagram of malarial attacks (represented by black triangles) in 17 volunteers with Chesson strain vivax malaria. The first four subjects were controls; the next five were given suppressive medication (0.3 gm. per week) for six months; the last eight were given suppressive medication for 12 months. All acute attacks were interrupted with quinine sulfate, 2 grams per day for 14 days.

long intervals of negative blood smears between the last attack and the end of the observation period in most of the subjects suggest that relapse activity was past.

Plasma drug levels: Plasma concentrations of chloroquine taken six hours after medication (primarily to provide assurance of drug ingestion) showed levels ranging from 10 to 127 micrograms per liter. Individual means ranged from 36 $\pm$ 0.9 to 50 $\pm$ 1.4. There was no progressive rise with continued medication. In more than half of 92 determinations made seven days after dosage with chloroquine, levels were 10 micrograms per liter or less. Fall-away was as rapid after the 27th and 53rd doses as after the 3rd medication.

Chlorguanide levels, determined three hours after each dose, ranged from 40 to

540 micrograms per liter of plasma. Patient means ranged from 170 ± 10.7 micrograms per liter to 260 ± 14.0 micrograms per liter. There was no progressive rise with continued medication and plasma levels fell below detectable limits in two to four days after dosage.

Toxicity: No toxic manifestations were noted during serial administration of either chloroquine or chlorquanide.

Complement fixation reactions: Positive complement fixation reactions with a *P. knowlesi* antigen (Rein *et al.*, 1948) appeared in four subjects while they were receiving suppressive medication. These men (S-17, S-27, S-28 and S-31) represented four of the nine men who were given weekly doses of chloroquine. None of the nine men who received chlorguanide showed positive reactions until after their delayed primary attacks. The positive reactions during suppression appeared 16 to 60 days after inoculation and remained elevated thereafter throughout the period of weekly medication.

DISCUSSION

It will be recalled that mild symptoms indicative of malaria occurred 10 to 12 days after inoculation in three of the subjects receiving chloroquine, but these subsided spontaneously and the regimens did not have to be altered. It is probable that these threatened breakthroughs would have been prevented by loading-doses or longer preliminary medication (cf. Packer, 1947). Another indication that there was recurrent subpatent erythrocytic parasitemia in the chloroquine-protected subjects (such as demonstrated by Fairley *et al.*, 1947) is the fact that 4 of these men developed positive malaria complement fixation tests.

The foregoing might suggest that chloroquine provided a smaller margin of safety against malaria than did chlorguanide. The fact, however, that delayed primary attacks occurred much later after chloroquine than after chlorguanide is an important consideration in favor of chloroquine, as it greatly increases the protection to be expected if occasional doses of drug are missed. Previous studies, such as those of Berliner *et al.*, (1948), Jones *et al.*, (1948), and Coatney *et al.*, (1948), have shown comparable latent intervals after therapeutic or suppressive administration of these drugs. The residual protection after chloroquine is usually explained by prolonged retention of the drug in the body, although in our experiment, plasma concentrations were below accurately measurable levels within one to two weeks after the last dose.

The effectiveness of both the chloroquine and the chlorguanide regimens in preventing patent malaria agrees with the results of field trials, such as those of Goldsmith (1946), Clark (1947), Ray (1948), Smith, Dy and Cabrera (1948), Elmendorf (1948) and Berberian and Dennis (1948). Against most strains of the malaria parasite, weekly doses of 0.3 gram of either drug appear adequate for suppression.

The effect of prolonged suppression upon the overall course of a vivax infection, i.e. upon the total number of acute attacks and upon the length of time from introduction of sporozoites to the final parasitic or clinical manifestations, is a matter of considerable interest. In our experiment, suppression for 6 or 12 months neither eradicated nor outlasted the underlying fixed-tissue infection. Relapses after quinine

treatment of the first delayed attacks came almost as soon as after the undelayed primary attacks in the controls, so that at first it appeared that the protected subjects might have to experience 12 to 15 months of active malaria after suppression had been stopped. However, attacks in the temporarily-protected subjects soon became widely spaced so that in the final analysis the total duration of their disease (from inoculation to final attack) was only slightly longer than in the controls, and they experienced fewer attacks per man. Although the terminal observation periods were not long enogh to provide dramatic proof of these points, it can be noted (table 2 and figure 1) that the final latent intervals in most subjects were sufficiently long to indicate infections were drawing to an end, and that the protected patients actually experienced less acute malaria.

The extremely interesting studies on malaria incidence in the 147th Infantry Regiment, which were continued for nearly three years after exposure to malaria on Guadalcanal, suggest that quinacrine suppression reduced the total amount of clinical malaria experienced by the group (Baker and Platt, 1947). Unfortunately terminal followup in these men was also too short to portray clearly the total malaria experience of the group.

London *et al.* (1946) found that 150 days of quinacrine suppression, given after quinine therapy of acute attacks of Pacific vivax malaria, did not reduce the relapse incidence. They did not have an opportunity, however, to follow the ultimate course of infections in the two groups, which may be regarded as including many heavily-inoculated subjects with a longer-than-average relapse expectancy.

Because of differences in the behavior of different strains of *P. vivax*, and in the numbers of sporozoites inoculated, one should expect divergent results from different observers as to the ultimate effect of long-term suppression of naturally-acquired malaria in individuals not subject to repeated reinfection. We have unpublished data to indicate that the average number of acute attacks and the average duration of infection are greater after heavy inoculations than after light. One can predict that, in general, the heavier the intial inoculum the longer will be the period of suppressive medication required to reduce the incidence of malaria after the conclusion of suppression.

SUMMARY

1. Chloroquine 0.3 gram of base or chlorguanide 0.3 gram of base, given once weekly for six months or for one year, effectively suppressed Chesson strain *vivax* malaria in volunteers.

2. Acute attacks of malaria appeared in all subjects after suppression was discontinued, whether 6 months or 12 months after inoculation.

3. Except for delayed onset, the pattern of attack following chloroquine suppression did not differ significantly from that following chlorguanide.

4. Although the evidence was not conclusive, it appeared that fewer attacks of malaria occurred in the protected subjects than in the unprotected, and that the total time from inoculation to final acute attack of malaria was only slightly prolonged by the period of suppression.

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SUMARIO

1. Cloroquina o Cloroguanida en dosis de 0,3 gramos de base administradas una vez semanal durante 6 meses o durante un año produjeron supresión efectiva de la cepa Chesson de *vivax* en voluntarios.

2. Ataques agudos de malaria aparecieron en todos los sujetos cuando el tratamiento supresivo fué suspendido a los 6 meses o al año, segun el caso, después de la inoculación.

3. El cuadro clínico que siguió a la supresión por cloroquina, si se exceptúa una ligera demora en la ocurrencia del ataque con esta última, no se diferenció prácticamente del que siguió a la supresión por cloroguanida.

4. Aunque no se puede llegar a una conclusión definitiva se notó que ocurrieron menor número de ataques de malaria entre los sujetos protegidos que entre los no protegidos y que el período total de tiempo desde la inoculación hasta el ataque final agudo de malaria fué apenas prolongado por el período de la supresión.

STUDIES IN HUMAN MALARIA

XXIII. Acquired Resistance to Chlorguanide in the Chesson Strain of *PLASMODIUM VIVAX*

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Studies to determine whether or not *Plasmodium vivax* would acquire resistance to the action of chlorguanide (paludrine, proguanil) were begun in December 1948, soon after it was learned that Schmidt *et al.* (1949) and Hawking and Perry (1948) had achieved paludrine-resistance in the simian parasite, *P. cynomolgi*. It had been demonstrated earlier by Williamson *et al.* (1947a and 1947b), Bishop and Birkett (1947, 1948), Knoppers (1947) and Greenberg (1949), that *P. gallinaceum* readily acquired such resistance.

While our work was in progress Seaton and Lourie (1949) published a brief report showing that *P. vivax* can become chlorguanide-resistant. Our studies confirm this and indicate that whenever underdosage occurs there is the probability of development of resistant substrains.

METHODS

All subjects were white male volunteers, who were prisoners in the Federal Correctional Institution, Seagoville, Texas and who had no history of previous malaria.

Both blood- and sporozoite-induced infections were studied. When infections were initiated by the intravenous injection of parasitized blood, coagulation was prevented by minimal amounts of heparin and dilution was with physiological saline. Sporozoite-induced infections were initiated by the bites of infected *Anopheles quadrimaculatus* mosquitoes, used as described in previous reports (Coatney *et al.*, 1948).

Chlorguanide was given in commercially prepared tablets[1] or in capsules containing subdivided tablets or weighed amounts of powdered chlorguanide. Dosages are expressed in terms of the hydrochloride, which is 87 per cent base. Plasma concentrations were estimated by the method of Spinks and Tottey (1946).

EXPERIMENTS AND RESULTS

Sensitivity of the parent strain to chlorguanide. The Chesson strain of *Plasmodium vivax* is very responsive to small doses of chlorguanide. Earle *et al.* (1948) showed in standardized tests that 25 mgm. of chlorguanide base given over a 4-day period, i.e. an average of 6.25 mgm. per day, reduced parasitemia, and that in two of four subjects there was permanent eradication of erythrocytic parasites. They did not try smaller doses against this strain.

We have tested very small doses of chlorguanide in eight primary attacks of sporozoite-induced Chesson strain vivax malaria and found that as little as 1.5 mgm.

[1] Furnished through the courtesy of E. I. du Pont de Nemours and Co.

of salt per day produced temporary clearance of parasitemia and fever (figure 1). The description of a typical case follows:

Volunteer S-213 was infected on 17 September 1948 by bites of 10 infected mosquitoes. Parasitemia became patent on the 14th day; fever appeared on the 13th day. Treatment with chlorguanide 1.5 mgm. per day for 14 days was begun on the 3rd day

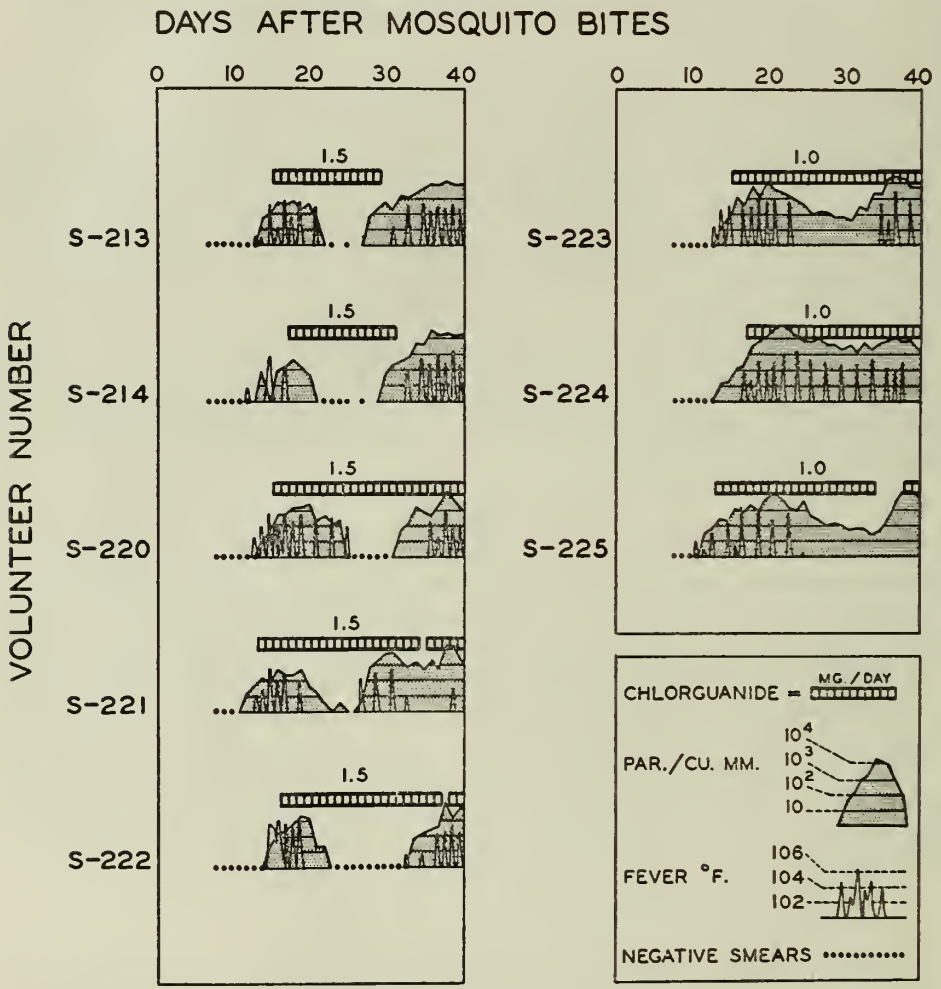

FIG. 1. The response of primary attacks of sporozoite-induced Chesson strain *vivax* malaria to small daily doses of chlorguanide.

of patency, when the parasite count was 700 per cu. mm. The patient was afebrile and smear-negative on 7th day of treatment, but parasites reappeared after a latent period of six days, and rose to 1200 per cu. mm. on the last day of therapy. The subject was then allowed to go untreated and exhibited 10 weeks of patent parasitemia.

Subjects S-214, S-220, S-221 and S-222 were also given 1.5 mgm. per day and

exhibited almost identical responses, i.e. there was temporary clearance of parasites and fever, followed by relapse while the same dosage was being continued. Three other subjects, S-223, S-224 and S-225, were given 1.0 mgm. per day; parasitemia was not temporarily cleared, although there was alleviation of fever in two of them. These 8 cases provide evidence that 1.5 mgm. of chlorguanide per day is approximately the minimal dosage able to reduce the number of erythrocytic parasites of Chesson strain *P. vivax* below the patent level, with resultant temporary amelioration of clinical malaria. The reappearance of parasites during treatment probably indicates the beginning of resistance to the drug.

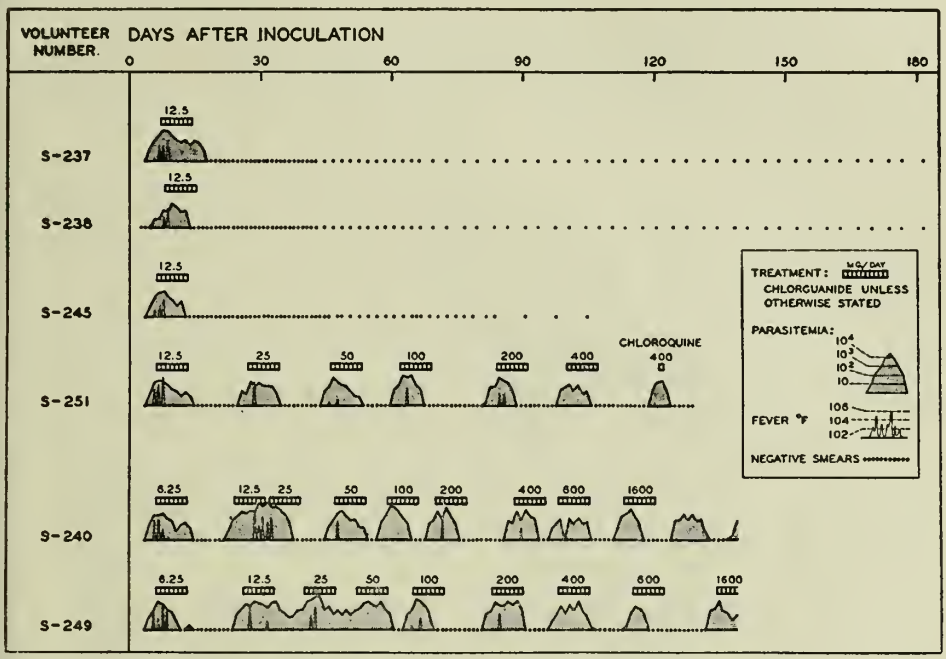

FIG. 2. The response of primary attacks of blood-induced Chesson strain *vivax* malaria, resulting from the intravenous inoculation of 0.5×10^6 parasites, to dosages of chlorguanide approximating the minimal amounts required for eradication of erythrocytic parasites.

For determination of the minimal quantity of drug required to *eradicate* erythrocytic parasites, each of six volunteers (figure 2) was given 500,000 erythrocytic parasites intravenously from a donor who had never received chlorguanide. Treatment was begun on the 3rd day of patent parasitemia. Four of the subjects (S-237, S-238, S-245 and S-251) were given 12.5 mgm. of chlorguanide per day for 7 days; in only one of the four did relapse occur. Two subjects (S-240 and S-249) were given 6.25 mgm. of chlorguanide per day for 7 days; relapses occurred in both. It appears that approximately 12.5 mgm. of chlorguanide per day for seven days is required to eradicate the erythrocytic forms of Chesson strain *P. vivax*.

Acquired resistance in blood passage strain. The first technique employed in our attempts to develop acquired resistance in blood-induced infections was similar to

that used by Schmidt *et al.* (1949). As acute attacks appeared in the subjects, dosages of chlorguanide were given which were sufficient to reduce the number or erythrocytic parasites but not sufficient to eradicate them. With each successive relapse the dosage was doubled, until the infection was either cured or until the maximal tolerated dosage was reached. Unless otherwise stated, the drug was always given daily for seven days. Nine volunteers were employed, inoculated over a period of six months. The course of the blood transfers is indicated in figure 3. The series began with S-227,

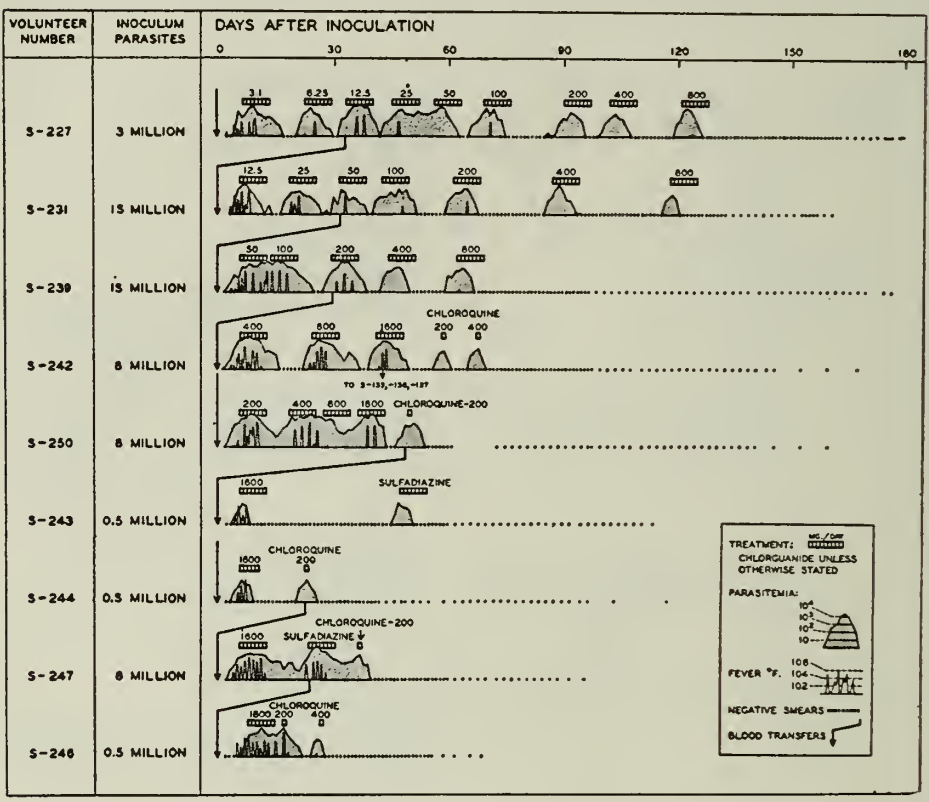

FIG. 3. Courses of therapy and subinoculations in nine volunteers used for the demonstration of acquired resistance to chlorguanide in blood-passaged Chesson strain *vivax* malaria. Unless otherwise stated therapy figures indicate milligrams of chlorguanide hydrochloride per day. Drug was given once daily except for 800 mgm and 1600 mgm amounts, which were given in divided doses four hours apart.

who received approximately 3×10^6 parasites from S-132, who had been infected by sporozoites. The first attack in S-227 was interrupted with 3.125 mgm. per day for seven days; dosages were doubled in successive relapses and cure was not obtained until the dosage had reached 800 mgm. (9th course of therapy). Subinoculation was made from S-227 to S-231 just prior to the former's 3rd course of therapy. S-231 was first treated with 12.5 mgm. but continued to have relapses until after 800 mgm. (7th course). Blood transfer was made to S-239 as shown; the latter's therapy began at 50 mgm. and likewise continued through 800 mgm. Transfers were made from

S-239 to S-242 and S-250. Volunteer S-242 experienced relapses after 400 mgm., 800 mgm. and 1600 mgm., then was cured by 400 mgm. of chloroquine (base) given in a single dose. S-250 experienced relapses after 200 mgm., 400 mgm., 800 mgm., and 1600 mgm. of chlorguanide and was then cured by 200 mgm. of chloroquine (single dose). Transfers were made from S-250 to S-243 and S-244, who were given only 500,000 parasites each, and their initial attacks were interrupted with 1600 mgm. of chlorguanide. Relapses occurred in both; blood was transferred from S-244 to S-247 (8×10^6 parasites), whose resultant infection was not even temporarily cleared by 1600 mgm. A transfer of 500,000 parasites to S-246 again produced an infection which was not affected by 1600 mgm. per day for seven days. Peak plasma concentrations during the 1600 mgm. per day courses ranged from 940 to 1560 micrograms per liter.

The amount of chlorguanide required for temporary interruption of Chesson strain *vivax* malaria was thus increased more than 1000-fold (> 1600 mgm. as compared with 1.5 mgm.).

In another series of infections (those beginning with the introduction of the parent strain in volunteers S-240, S-249 and S-251, see figure 2) the first course of therapy was 6.25 mgm. or 12.5 mgm., with subsequent doubling of the dosage. A sequence of relapses ensued, duplicating the series which had been intiated in S-227 and subsequent subinoculees. In one subject a dosage of 1600 mgm. was reached within four months. This indicates that it is not necessary to start with doses as low as 1.5 mgm. or 3.125 mgm. per day to begin the induction of drug-fastness, nor is subinoculation essential. We have had essentially similar sequences when treatment was with single doses of chlorguanide, doubled at each successive relapse. Apparently whenever the drug is given in amounts insufficient to destroy all erythrocytic parasites, the selective or adaptive process takes place.

Studies in sporozoite-induced infections. In four volunteers infected with the Chesson strain of *P. vivax* by the bites of 5 to 6 lightly infected mosquitoes, attempts were made to demonstrate chlorguanide resistance by prolonged suppression, with progressively increased doses of chlorguanide, begun during the primary attack and continued for 4 or 5 months. The initial doses (1.5 and 1.0 mgm. per day, as described in the paragraphs on sensitivity of the parent strain) were so low that the subjects experienced several weeks of patent parasitemia during the early stages of the infection. There was thus adequate opportunity for the acquisition of immunity to erythrocytic parasites. Although parasites became repatent in two subjects while they were receiving 100 mgm. of chlorguanide per day, whereas 1.5 mgm. per day had previously been suppressive, such responses can be explained by action on erythrocytic parasites alone. We do not know whether or not progressively increased underdosage exerts any direct selective effect upon fixed-tissue parasites.

In other tests, we have found that 200, 100 or even 50 mgm. of chlorguanide given once weekly will suppress Chesson strain *vivax* infections caused by the bites of either 30 or 3 heavily-infected mosquitoes. After the discontinuance of suppression, one year after exposure, malaria appeared in all of the heavily-infected, and in most of the lightly-infected subjects. The resultant acute attacks in all instances were amenable to therapy with single 100 mgm. doses of chlorguanide, which resulted in parasite clearance. Intervals to first relapse were quite similar to those in the parent

strain. Such evidence indicates that prolonged exposure of sporozoite-induced infections to small doses of chlorguanide, provided they are sufficient to suppress erythrocytic forms at all times, does not induce a significant degree of resistance.

Susceptibility of chlorguanide-resistant substrain to other drugs. Five subjects with relapses resulting from the chlorguanide-resistant substrain were treated with chloroquine in dosage of 0.2 gram of base (single dose), an amount which usually produces permanent cure of trophozoite-induced Chesson strain *vivax* malaria. In each of the five patients it cleared patent parasitemia; in three of the five it produced cures. In the other two subjects cures were obtained when dosage was raised to 0.4 gram (single dose). The chlorguanide-resistant substrain thus is normally susceptible to chloroquine.

In two subjects sulfadiazine in dosage of four grams daily for seven days was used therapeutically against the chlorguanide-resistant strain because of the observation by Greenberg (1949) that a chlorguanide-resistant strain of *P. gallinaceum* was highly susceptible to sulfadiazine. No clear-cut evidence of increased susceptibility was obtained in our two subjects (figure 3).

Immunologic identity of the resistant substrain and the parent strain. Although there was no reason to suspect that the metabolic changes involved in the acquisition of drug-fastness would change the antigenic properties of the strain, limited observations bearing upon this point were made. Subinoculations of 10×10^6 parasites from volunteer S-242, taken just prior to therapy with 1600 mgm. of chlorguanide, were made into each of three subjects, S-135, S-136, and S-137, on the 526th day of the latters' sporozoite-induced infections. The recipients had previously experienced from 115 to 155 days of patent parasitemia and each had shown his immunity to the Chesson strain by an ability to clear parasitemic relapses promptly, without therapy. Daily blood smears for 16 days after the subinoculations did not reveal any parasitemia in the recipients, whereas similar inoculations in non-immune subjects produce patent malaria within two to three days. Although fragmentary, these data indicate that no drastic antigenic change is associated with the acquisition of chlorguanide resistance.

Attempts to induce resistance to chloroquine. In a series of three subjects, attacks of trophozoite-induced Chesson strain vivax malaria were treated with single subcurative doses of chloroquine, beginning with 50 mgm. of base. Subinoculations were made at the time of the first relapse and successive relapses were treated with doubled doses of chloroquine. No further relapses occurred after therapy with 0.2 gm., which is approximately the threshold dose for eradication of erythrocytic parasites of the Chesson strain. In four other subjects with blood-induced infections, relapses after maximum tolerated dosage of chlorguanide were interrupted with 200 mgm. of chloroquine. Further relapses occurred in two, but the infections were permanently eradicated with 400 mgm. The technique of subcurative dosage, successively doubled as described for chlorguanide, has thus not been successful in bringing about resistance to chloroquine even when subinoculations were employed.

DISCUSSION

The most important conclusion from the foregoing studies is that the erythrocytic parasites of *Plasmodium vivax* readily become resistant to chlorguanide when sub-

jected to dosages below that required to eradicate them. This was not an occasional occurrence, but happened invariably. Although serial subinoculations were employed in an attempt to speed up the process, they were not an essential part of the procedure.

It is unfortunate that no direct evidence was obtained as to whether or not progressively increased doses of drug can produce drug-resistance by direct action upon exoerythrocytic parasites. Our attempts to study this were inconclusive because of the ease with which erythrocytic parasites develop drug-resistance and the fact that the host develops immunity to such erythrocytic forms. It has of course been established (Seaton and Lourie, 1949; Schmidt, *et al.* 1949; and others) that fixed-tissue parasites can transmit drug-resistance which has been acquired during the erythrocytic phase.

One can only speculate as to the process through which successive generations of erythrocytic parasites acquire such complete insensitivity to chlorguanide. In vivax malaria, the facts are perhaps more compatible with the selection of spontaneous or induced mutants, or with an actual adaptive process in individual parasites, than with the selection of parasites which were highly resistant from the beginning. If every large population of parasites had in it individual members which were capable of surviving in spite of large doses of chlorguanide, one would expect a high incidence of relapse in all blood-induced infections after intensive chlorguanide therapy. This does not occur, however, until after a selective or adaptive process has taken place.

The practical implications of acquired drug-fastness are obvious, although we do not know to what extent such strains might develop in the field. One may conjecture that in an area where chlorguanide was widely used, the chances for acquired resistance would be excellent. The chain of events might begin with an inadequately treated case; 100 mgm. as a single dose is perilously near the subeffective level for many strains. It could also begin during suppressive regimens. While our work with the Chesson strain does not indicate that prolonged suppression after a single exposure is necessarily conducive to drug-resistance, in actual practice there would be haphazard dosage and repeated exposures, sometimes perhaps to strains which were naturally less susceptible to chlorguanide. Occasional mosquito bites might be so timed with relation to the taking of drug that erythrocytic parasites would be exposed to subeffective concentrations. It would seem inevitable that chlorguanide-resistant strains would appear under such conditions. To reduce the chance of this, chlorguanide should always be given in adequate dosage, and it should not be the sole reliance in any area.

SUMMARY

By progressively increased subcurative regimens in blood-induced infections, with serial subinoculations, a substrain of Chesson strain of *Plasmodium vivax* was developed which resisted treatment with 1600 mgm. of chlorguanide daily for seven days. This is more than 1000 times the amount of drug which will produce a temporary alleviation of infections in the parent strain, and 128 times the amount which permanently eradicates erythrocytic infections in the parent strain. A similar technique did not produce resistance to chloroquine.

Studies in sporozoite-induced infections, although less conclusive, likewise pro-

duced evidence of drug-resistance, which could be satisfactrily explained by action upon erythrocytic parasites alone.

Chlorguanide-resistant strains responded normally to chloroquine.

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SUMARIO

Casos de infecciones inducidas por una serie de sub-inoculaciones de sangre sometidas a un régimen de tratamiento con dosis subcurativas de cloroguanida desarrollaron una sub-cepa de la cepa Chesson de *Plasmodium vivax* que resistió a un tratamiento de 1600 mgm. de cloroguanida diarios durante 7 días. Esta cantidad es más de 1000 veces mayor que la cantidad de droga necesaria para producir un alivio temporal en infecciones de la cepa madre y 128 veces la cantidad necesaria para erradicar permanentemente infecciones eritrocíticas de la misma cepa madre. Una técnica similar no produjo resistencia a la cloroquina.

Estudios en infecciones inducidas por esporozoítos, aunque menos concluyentes mostraron también resistencia a la droga, la cual pudiera ser explicada satisfactoriamente por una acción específica contra los parásitos en los eritrocítos únicamente.

Las cepas resistentes a la clorogúanida responden normalmente a la cloroquina.

STUDIES IN HUMAN MALARIA

XXIV. Protective and Therapeutic Trials of SN 10,751 (Camoquin) against the Chesson Strain of *PLASMODIUM VIVAX*

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SN 10,751, or 7-chloro-4-(3-diethylaminomethyl-4-hydroxyanilino) quinoline (also known as amodiaquin, camoquin, miaquin and CAM-AQ1) (figure 1), was among the 4-aminoquinoline derivatives studied during the World War II program of antimalarial drug testing, and many of its properties have been summarized in the "Survey of Antimalarial Drugs, 1941–1945" (Wiselogle, 1946) and by Berliner *et al.* (1948). Although the compound was shown by Butler (in Wiselogle, 1946) to be three to four times as active as quinine against the erythrocytic forms of McCoy strain *Plasmodium vivax*, no appraisal was made of its possible prophylactic or curative activity against sporozoite-induced infections. In this paper we will describe such tests against standardized infections of Chesson strain *vivax* malaria.

SN 10,751 has been subjected to field trials in Brazil (Penido *et al.*, 1947, Mein *et al.*, 1947); in Egypt (Halawani *et al.*, 1947, 1948); in India (Simeons and Chhatre, 1947); and in the Philippines (Ejercito and Duque, 1949). All have pointed to its being an active and relatively non-toxic antimalarial.

MATERIALS AND METHODS

The procedures used in these experiments have been those previously described for standardized trials of other antimalarial drugs by Coatney *et al.* (1948). The patients were white male volunteers at the Federal Correctional Institution, Seagoville, Texas, whose participation was made possible by authorities of the Bureau of Prisons of the Department of Justice. Each man was infected with the Chesson strain of *P. vivax* by the bites of *Anopheles quadrimaculatus* mosquitoes, which had been allowed to feed approximately two weeks earlier upon suitable gametocyte carriers. After a mosquito had fed upon three volunteers in turn, it was dissected and the number of sporozoites in its salivary glands was graded on a scale of 0 to 4+.

Whenever possible, subjects were kept under observation for 18 months, with blood smears at least twice weekly. All malarial attacks during that period were carefully studied with temperature records every four hours and daily parasite estimations. Therapy was given during each acute attack, the first dose of drug being given not later than the 5th day of patent parasitemia.

SN 10,751 was given as the dihydrochloride dihydrate (76.6 per cent base), in tablets which contained 50 mgm. of base.[2] Other patients in the same experiment

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[2] Supplied through the courtesy of Parke, Davis and Company.

were treated with chloroquine diphosphate, in tablets containing 100 mgm. of the base.

Plasma concentrations of SN 10,751 and its degradation products, after hydrolysis in an alkaline medium, were measured by the irradiation method of Brodie *et al.* (1947).

EXPERIMENTS AND RESULTS

Protective tests. The activity of SN 10,751 as a protective agent was tested when it was given (1) on the day before, the day of and for six days after exposure to infective mosquitoes and (2) once weekly beginning four to six days before, and continued for six weeks after exposure. The two tests were conducted simultaneously, using 14 volunteers, numbered S-116 through S-129. Six subjects were infected on 6 November 1947 and eight on 16 December 1947. Drug regimens and sporozoite inocula are indicated in table 1. The four controls developed acute malaria promptly, 11 to 13 days after exposure. The five subjects given SN 10,751 for six days after exposure did

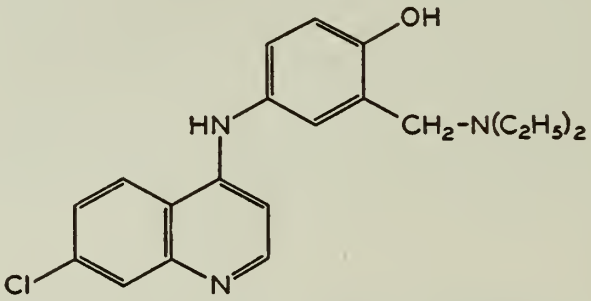

FIG. 1. Structural Formula of SN 10,751 (Camoquin)

not have patent malaria until 53 to 102 days (mean 69 days) after the last dose of drug, corresponding to 59 to 108 days after mosquito bites. The five subjects given 0.3 gram once weekly did not have any evidence of malaria during the six weeks of drug administration, but parasites appeared 28 to 41 days (mean 34.2 days) after the last dose, or 63 to 76 days after mosquito bites.

Therapeutic trials. The 14 volunteers in the preceding experiment were assigned in advance to therapy with either SN 10,751 or chloroquine. The dosage for both drugs was 0.1 gm. every six hours on the first day, followed by 0.05 gm. every six hours for 13 days, i.e. 3.0 grams in 14 days. The only exceptions were S-124 and S-126 who were treated with another drug, febrifugine, during their first two attacks; S-121 who received one course of chloroquine instead of SN 10,751 by error; and S-128, who was given curative therapy with isopentaquine and quinine after his first attack, because of the discovery of diabetes mellitus. In all, 32 attacks were interrupted with SN 10,751 and 31 with chloroquine. Details of the responses are summarized in tables 2 and 3. Due to the complicating factor of acquired immunity in later attacks, only the first three attacks in each subject will be analyzed with respect to parasite clearance, clinical response, incidence of relapse and intervals from treatment to relapse.

SN 10,751 cleared parasitemia promptly, though slightly less rapidly than chloroquine. In 18 attacks, blood smears were still positive in 12 (67 per cent) at 48 hours after the first dose of drug, in seven (39 per cent) at 72 hours, three (17 per cent) at 96 hours and in none at 120 hours. Mean clearance time was 3.2 days. Nineteen parallel cases treated with the same amount of chloroquine showed seven (37 per cent) still positive at 48 hours, five (26 per cent) at 72 hours and none at 96 hours. Mean clearance time was 2.5 days. These clearances were more rapid than in attacks treated with large doses of quinine sulfate (2 grams of base daily for 14 days) or

TABLE 1

The protective effect of SN 10,751 against the Chesson strain of Plasmodium vivax

DRUG REGIMEN	SET NO.*	VOLUNTEER		INFECTED MOSQUITOES PER MAN ON DAY 0		LAST PROTECTIVE DRUG (DAY AFTER BITES)	FIRST PATENT PARASITEMIA (DAYS AFTER BITES)	FIRST FEVER OF 101°F. (DAYS AFTER BITES)
		Code No.	Weight kg.	Number	Sum of plusest			
SN 10,751, 0.6 gram on day before bites, then 0.3 gram per day through 6th day after bites (1-1-6 regimen)	1	S-116	77	8	14+	6	108	110
	2	S-117	69	3	4+	6	70	180‡
	3	S-122	66	10	31+	6	61	64
	4	S-123	73	10	33+	6	77	81
	5	S-124	70	10	35+	6	59	63
SN 10,751, 0.3 gram once weekly, begun 6 or 4 days before bites and continued for 6 doses after bites	1	S-118	77	9	16+	35	76	79
	2	S-119	86	3	4+	35	63	68
	3	S-125	77	10	31+	38	69	71
	4	S-126	74	10	33+	38	76	79
	5	S-127	64	10	35+	38	71	157‡
Controls, no protective drug	1	S-120	68	9	16+	—	13	14
	2	S-121	73	3	4+	—	13	12
	3	S-128	70	10	31+	—	11	11
	4	S-129	61	10	34+	—	11	10

* Patients with corresponding set numbers were bitten by the same mosquitoes.

† Sporozoite density in each infected mosquito was graded on a scale of 1+ to 4+.

‡ First fever occurred during second attack of malaria.

chlorguanide (1 gram per day for 10 or 14 days), where the means were 3.7 and 5.4 days, respectively (figure 2).

Clinical responses, measured in terms of the time of the last fever of 101° F. or more, were nearly the same in the SN 10,751 and chloroquine groups. Of the 18 attacks interrupted with the former drug, 16 were febrile. In seven of these (44 per cent) there was no further fever after onset of therapy, in 15 (94 per cent) the last fever occurred within the first 24 hours after treatment began, and in none was there fever later than 32 hours after the first dose of drug. In the 19 cases treated with chloroquine, 13 were febrile. In nine of these (69 per cent) there was no further fever after onset of treatment; in eleven (85 per cent) the last fever occurred in the first 24 hours after onset of treatment; and all were afebrile by 44 hours after the first dose of chloroquine.

TABLE 2

Summary of 32 attacks of Chesson strain vivax malaria interrupted with SN 10,751 (Camoquin) in dosage of 0.4 gram of base on the first day followed by 0.2 gram per day for 13 days, i.e., three grams in 14 days

VOLUNTEER NO.	ONSET OF PATENCY CURRENT ATTACK (DAY AFTER BITES)	PARASITES/5000 WBC ON FIRST DAY OF THERAPY	DAYS FROM FIRST DOSE OF DRUG UNTIL:		DAYS FROM LAST DOSE OF DRUG UNTIL:	
			Smear negative	Afebrile*	Repatency	End of observation if no relapse
			First attacks			
S-118	76	260	2	0	55	—
S-120	13	300	2	1	81	—
S-121	13	2120	4	1	45	—
S-123	77	4540	3	1	78	—
S-125	69	5030	4	2	39	—
S-129	11	1020	4	1	46	—
			Second attacks			
S-118	148	1140	2	0	96	—
S-120	110	3170	2	1	64	—
S-123	173	40	2	0	80	—
S-125	125	390	3	1	45	—
S-129	73	820	3	0	52	—
			Third attacks			
S-118	262	1160	5	0	93	—
S-120	192	1160	3	0	111	—
S-121	149	5180	5	0	58	—
S-123	269	550	2	—	92	—
S-124	172	380	3	—	71	—
S-125	188	20310	4	1	44	—
S-129	141	120	5	1	56	—
			Fourth attacks			
S-118	370	4100	3	1	—	158
S-120	319	150	2	—	145	—
S-121	224	1120	4	0	68	—
S-123	379	810	2	—	—	150
S-124	261	0†	0	—	100	—
S-129	214	1380	3	—	73	—
			Fifth attacks			
S-120	482	1630	3	1	—	50
S-121	309	4360	3	0	69	—
S-124	379	30	1	—	99	—
S-129	305	190	1	—	143	—
			Sixth attacks			
S-121	395	3430	3	1	85	—
S-124	519	90	1	—	—	10
S-129	466	0†	0	—	—	62
			Seventh attack			
S-121	497	420	3	—	—	34

* Dash indicates an afebrile attack; 0 indicates a febrile attack in which no fever (101°F)occurred after beginning of treatment; 1 indicates that last fever occurred during first 24 hours of treatment, etc.

† Parasitemia cleared spontaneously before treatment was begun.

TABLE 3

Summary of 31 attacks of Chesson strain vivax malaria interrupted with chloroquine in dosage of 0.4 gram of base on the first day, followed by 0.2 gram per day for 13 days, i.e., 3 grams in 14 days

These cases were concurrent with those shown in Table 2.

VOLUNTEER NO.	ONSET OF PATENCY CURRENT ATTACK (DAY AFTER BITES)	PARASITES/5000 WBC ON FIRST DAY OF THERAPY	DAYS FROM FIRST DOSE OF DRUG UNTIL:		DAYS FROM LAST DOSE OF DRUG UNTIL:	
			Smear negative	Afebrile*	Repatency	End of observation if no relapse
			First attacks			
S-116	108	460	2	2	73	—
S-117	70	520	2	—	88	—
S-119	63	3670	3	0	61	—
S-122	61	510	2	1	65	—
S-127	71	1300	4	—	64	—
S-128	11	970	4	2	50	—
			Second attacks			
S-116	198	1280	4	0	86	—
S-117	176	2960	2	0	81	—
S-119	143	610	2	—	69	—
S-121	74	3010	3	0	57	—
S-122	143	670	2	1	71	—
S-127	153	10370	2	0	60	—
S-128	78	970	3	2	—†	—
			Third attacks			
S-116	302	0‡	0	—	141	—
S-117	275	320	2	0	76	—
S-119	230	300	2	0	261	—
S-122	232	200	2	—	72	—
S-126	173	1050	4	—	63	—
S-127	231	12250	2	0	68	—
			Fourth attacks			
S-116	461	4380	2	1	—	56
S-117	367	1380	3	0	133	—
S-119	509	320	2	—	—	7
S-122	322	20	1	—	—	86
S-126	256	860	3	—	69	—
S-127	316	8250	3	0	85	—
			Fifth attacks			
S-117	517	1220	3	—	—	14
S-126	343	460	2	—	76	—
S-127	417	4270	2	0	87	—
			Sixth attacks			
S-126	437	790	1	—	77	—
S-127	520	3030	3	—	—	9
			Seventh attack			
S-126	532	130	1	—	—	0

* Dash indicates an afebrile attack; 0 indicates a febrile attack in which no fever (101°F) occurred after beginning of treatment; 1 indicates that last fever occurred during first 24 hours of treatment, etc.

† Treated with isopentaquine and quinine before next relapse.

‡ Parasitemia cleared spontaneously before treatment was begun.

Relapses occurred in all instances where first, second or third attacks were interrupted with SN 10,751 or chloroquine. The mean interval from treatment to relapse in the 18 such attacks treated with SN 10,751 was 67.0 ± 5.06 days; while the cor-

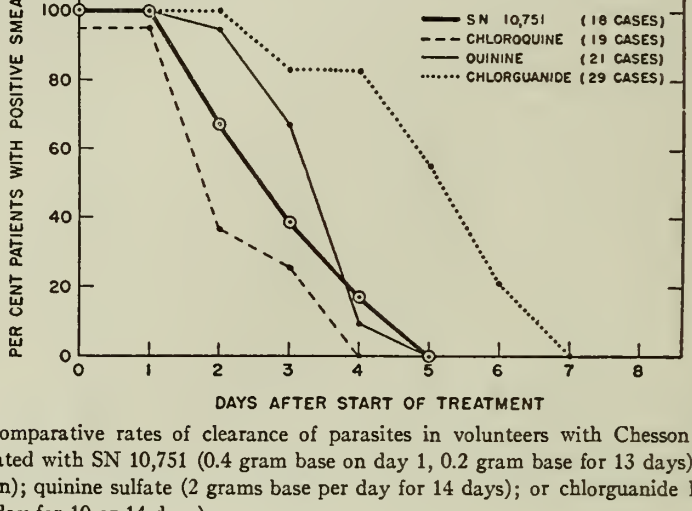

FIG. 2. Comparative rates of clearance of parasites in volunteers with Chesson strain vivax malaria, treated with SN 10,751 (0.4 gram base on day 1, 0.2 gram base for 13 days); chloroquine same regimen); quinine sulfate (2 grams base per day for 14 days); or chlorguanide hydrochloride 1 gram per day for 10 or 14 days).

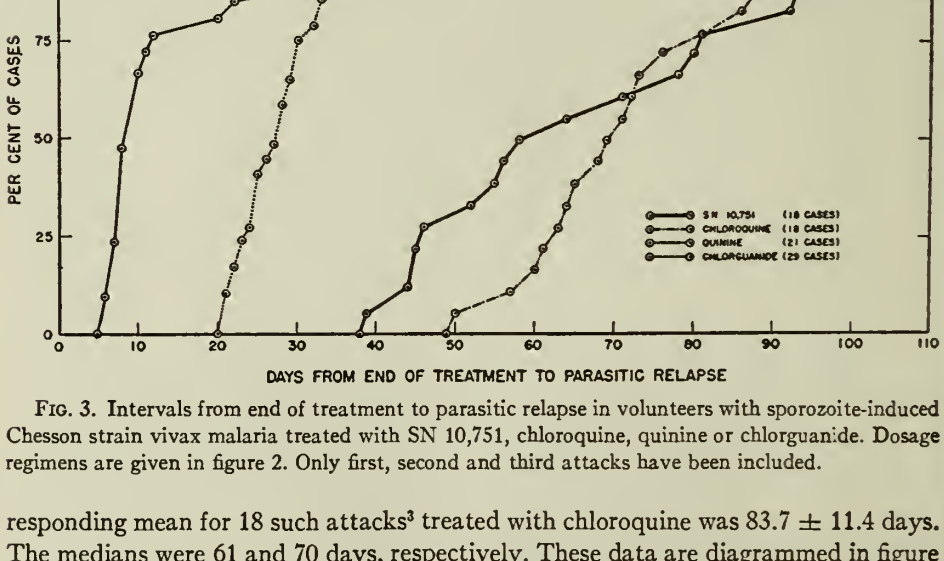

FIG. 3. Intervals from end of treatment to parasitic relapse in volunteers with sporozoite-induced Chesson strain vivax malaria treated with SN 10,751, chloroquine, quinine or chlorguanide. Dosage regimens are given in figure 2. Only first, second and third attacks have been included.

responding mean for 18 such attacks[3] treated with chloroquine was 83.7 ± 11.4 days. The medians were 61 and 70 days, respectively. These data are diagrammed in figure

[3] One subject treated with chloroquine during his first attack was given curative therapy prior to relapse and has been omitted from this calculation.

3 with corresponding data for quinine sulfate and chlorguanide, where the median intervals from treatment to relapse were 10 and 28 days, respectively.

Five subjects were treated with SN 10,751 during each of their attacks and had no other therapy. Three of these had four attacks each, one had five attacks and one had six attacks, during 18 months of observation. Five subjects treated with chloroquine had exactly the same incidence of attacks; three had 4 attacks each, one had five attacks and one had six attacks.

Toxicity: Three of the five patients on the protective regimen of 0.3 gm. of SN 10,751 per day complained of nausea, and one vomited several times on the 8th day of drug administration. During the 32 therapeutic courses of 0.2 gm. per day there were no complaints, other than those attributable to malaria. Blood counts and urinalyses were normal.

Blood levels. The procedure used for quantitative estimation of SN 10,751 also measures fluorescent metabolic products of that drug, which is rapidly degraded in the human body. The prolonged retention of these degradation products, which are presumably antimalarial, is indicated by the fact that detectable, though not accurately measurable quantities, could often be found in the blood plasma as long as 30 to 40 days after the last of seven weekly doses, and for 40 to 50 days after courses of therapy. Concentrations during and after the periods of therapy were subject to many erratic and unexplained variations. In general, levels were lower than those obtained with the same doses of chloroquine.

SUMMARY AND CONCLUSIONS

SN 10,751 proved to be a very effective drug for suppressing and alleviating Chesson strain *vivax* malaria. It compared favorably with chloroquine, in that it was an effective suppressant when given in dosage of 0.3 gram of base once weekly, it terminated acute attacks promptly and delayed relapses for at least six to seven weeks after therapy. It was well tolerated in dosages of 0.2 gram of base per day for 14 days[4] and 0.3 gram per day for eight days. Like other 4-aminoquinolines, it displayed activity only against erythrocytic parasites. Large doses administered for six days after exposure did not prevent establishment of infections, nor did they alter subsequent patterns of infection in the temporarily-protected subjects. The delay of infection following such short administration is best explained by delayed elimination of active metabolic products of the drug, which similarly delay relapse after therapy of acute attacks. There was no evidence of any ability to prevent relapses.

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[4] This therapeutic dosage regimen was chosen because an administration period of 14 days is our routine in curative trials. The properties of SN 10,751 are such that it should be effective in interrupting acute malaria in dosages equivalent to those of chloroquine, i.e. 1.5 grams of base given over a period of 4 days. Single doses have been shown to be therapeutically effective; the same is true for chloroquine.

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SUMARIO Y CONCLUSIONES

SN 10.751 probó ser una droga muy efectiva tanto para supresión como para alivio contra la cepa Chesson de malaria a *vivax*. Se compara favorablemente con cloroquina en el sentido de que fué un supresivo efectivo duando se administró en dosis de 0,3 gramos de base una vez semanal terminando rápidamente con los ataques agudos y dilatando las recaídas por lo menos de 6 a 7 semanas después de la terapéutica. Fué bien tolerado en dosis de 0,2 gramos de base diarios durante 14 días° y 0,3 gramos diarios durante 8 días. Al igual que otras 4-aminoquinolinas desplegó actividad solamente contra los parásitos en los eritrocitos. Grandes dosis administradas después de 6 días de la exposición no impidieron el establecimiento de la infección ni alteraron el cuadro clínico subsiguiente en los sujetos temporalmente protegidos. La demora de la infección subsiguiente a estas administraciones de tipo corto es explicada por una demora en la eliminación de los productos metabólicos activos de la droga lo que igualmente demora las recaídas posteriores a la terapéutica de ataques agudos. No se notó ninguna capacidad para prevenir las recaídas.

COMPLEMENT FIXATION WITH *PLASMODIUM KNOWLESI* ANTIGEN FOR MALARIA DIAGNOSIS[1]

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The 1938–39 papers of Coggeshall et al. (1938) and Eaton et al. (1939) stimulated considerable interest in the complement fixation test for malaria (Stratman-Thomas and Dulaney, 1940), (Dulaney et al., 1942). This interest increased during the war years because of the high incidence of malarial infection in men who had served in the Southwest Pacific area (Lippincott et al., 1945), (Dulaney and Watson, 1945). The chronic relapsing malaria seen in these veterans differed in several respects from the usual malarial infection and sporozoite induced malaria, namely, in the repeated innoculations of sporozoites from infected mosquitoes, more numerous relapses, and the absence of a prolonged latent period. While relapses occurred in all groups it is reasonable to suppose that men from this area would have a higher degree of residual infection readily demonstrable by complement fixation.

The close correlation between initial parasitemia and positive serological reaction indicated the value of the complement fixation test in early diagnosis of naturally acquired malaria (Dulaney et al., 1942). Furthermore a positive serological reaction has been shown to persist long after parasites are no longer demonstrable in blood films (Dulaney and Watson, 1945), (Rein and Kent, 1945). This serological reaction, if present two years or more after the last exposure would be additional proof of the effectiveness of the complement fixation test in detecting malaria of long standing. For this reason the tests were made at Kennedy Veterans Hospital in the years 1947 and 1948.

METHODS AND MATERIALS

A total of 204 complement fixation tests for malaria was carried out on sera from 174 male patients, hospitalized 190 times. The patients included seven negroes. The ages ranged from 17 to 59 years. While all major theaters of the war were represented the 116 veterans of the Southwest Pacific Theater constituted the majority. Thirty-four were from areas in which there was little or no exposure to malaria. Of the 44 patients admitted 52 times with either demonstrable parasitemia or positive complement fixation reaction, or both, two were in the China Burma India Theater, one was in the Mediterranean and one was exposed in an endemic area of the United States. The patients were hospitalized for malaria, for "rating examinations for malaria," for other febrile diseases and for splenomegaly.

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Thick and thin blood films were obtained on all patients. If parasites were not demonstrated in the initial smears a subsequent series of three to six blood films were examined. Either Wright's or Field's stain was used. Blood samples were collected at various times in relation to parasitemia for the complement fixation tests which were carried out at the University of Tennessee Medical School. A phosphate buffer extract of *P. knowlesi* parasites (Dulaney and Morrison, 1944) was used in tests employing four 50 per cent units of complement. While various types of malarial parasites have been used as sources of antigens, the greatest sensitivity and specificity has been obtained from such *P. knowlesi* preparations (Dulaney et al., 1942).

All sera were tested in dilutions ranging from 1:5 to 1:160. Any reaction of 4+ or greater was recorded as positive. The results of routine tests for syphilis were also tabulated for all patients and correlated with the results of the complement fixation tests for malaria.

TABLE 1

Results of complement fixation tests with sera from patients with long standing malaria

GROUP	NUMBER OF PATIENTS	NUMBER OF ADMISSIONS	POSITIVE	RESULTS DOUBTFUL	NEGATIVE
I. Demonstrable parasitemia, *P. vivax*..	28	35 (42)*	31 (38)	3 (3)	1 (1)
II. History of malaria within one year without parasitemia.....	80	89 (90)	17 (18)	10 (10)	62 (62)
III. History of malaria one year or more before examination.....	37	37 (40)	0	6 (7)	31 (33)
IV. No history of malaria or exposure..	29	29 (32)	0	4 (4)	25 (28)
Totals.....	174	190 (204)	48 (56)	23 (24)	119 (124)

* Figures in parentheses indicate the number of tests.

RESULTS

The classification was based upon the patient's story of his illness and exposure to malaria, and was confirmed in most instances by service records.

The grouping of the patients and the results of blood film examinations and complement fixation tests are presented in Table 1.

Group I. Patients with demonstrable parasitemia, P. vivax.—This group included 28 patients with records of 35 hospital admissions. The close correlation between parasitemia and complement fixation tests is shown by the 31 (89 per cent) positive serological reactions. There were three doubtful reactions and one negative.

Group II. Patients with history of malaria within one year but without demonstrable parasitemia.—The 80 patients comprising this group had a record of 89 hospital admissions. The serological data included 17 positive, 10 doubtful and 62 negative reactions. It is obvious verified positive reactions in this group would offer the greatest value in diagnosis. In 11 instances the positive reaction followed chemotherapy, in four no chemotherapy was received before admission, and in two no definite statement was recorded.

Group III. Patients with history of malaria one year or more before examination.—The results were negative in the 37 patients of this group.

Group IV. Patients with no history of malaria or exposure to malaria.—In 25 of 29 patients the results were negative. However the remaining four gave doubtful reactions in serum dilutions of 1:5 and 1:10, with clinical diagnosis of brucellosis, pyrexia of unknown origin, psychoneurosis, and choledocholithiasis. The serological reaction in the *Brucella suis* infection was 3+ in 1:5 and 2+ in 1:160 dilutions of serum. In the latter patient later tests were negative.

Since false positive serological reactions for syphilis have been reported in malaria, routine tests were made on certain patients. These included the Kahn and cardiolipin flocculation tests, and the Kolmer and cardiolipin complement fixation tests. The results were positive in three of 22 patients with both demonstrable parasitemia and positive complement fixation tests for malaria but negative in the 14 with no demonstrable parasitemia. In 130 patients with doubtful or negative complement fixation tests for malaria, three (2.3 per cent) gave a positive serological reaction for syphilis. Rein and Kent (1947) reported similiar results.

DISCUSSION

While the complement fixation test is inadequate for use in prognosis and control of malaria, further use of the test as a diagnostic measure is suggested by the results in Group I. In this group of 35 admissions of patients with parasitemia, there were 31 (89 per cent) with positive serological reactions. These results are similiar to the findings in naturally acquired malaria of Dulaney, Stratman-Thomas and Warr (1942) who reported 82 per cent with positive blood films were also positive serologically. In a later study on chronic relapsing malaria, Dulaney and Watson (6) found the incidence of positive complement fixation tests to be six times that of coincident blood film examinations. The sensitivity of the serological test is emphasized by the fact that 94 per cent of the patients were positive at some time during the study. Rein, Kent et al. (1949) in studies of patients infected with St. Elizabeth strain of *P. vivax* reported 195 positive complement fixation reactions in 199 malarial attacks. The serological reaction became positive 7.2 days after the primary attack and persisted for an average of 42 days. In repeated late relapses the reaction appeared earlier, 4.4 days after parasitemia, and persisted 125 days. During long periods of latency the positive reaction disappeared.

Probably the most significant findings are in Group II. which consists of patients with a history of an attack of malaria within a year but no demonstrable parasitemia. In 17 the serological reaction was the only laboratory confirmation of the patient's history. Dulaney and Watson, whose unpublished data are being cited again, found 40 of 261 patients with positive serological reactions and negative blood films. The results in Group II. are of interest in evaluating the complement fixation test as a diagnostic aid in malaria of long standing, such as is seen in the group of veterans studied.

The patients, in whom the positive complement fixation test was the only laboratory confirmation of the clinical diagnosis, represent 9 per cent of the total of 190

studied, a not inconsiderable part of a representative group which might present itself to a hospital such as Kennedy Veterans Hospital.

The doubtful reactions seen in Group IV. suggest the possibility of false positive serological reactions other than syphilis. Since these reactions may be due in part, to remote exposure to malaria or other causes, the tests should be done where malaria is endemic as well as in other areas.

SUMMARY

A series of complement fixation tests for malaria were carried out with sera from 174 patients and correlated with blood film examinations. Of these men 145 had a history of malaria or exposure to malaria, and 106 had served in the Southwest Pacific area. The serological reactions were positive in 31 of 35 admissions in which parasitemia was demonstrable. In contrast, the serological reactions were all negative in that group with no history of malaria for more than a year before examination.

Of 89 admissions in which parasitemia was not demonstrable although the patients had histories of malarial attacks within the year before examination, positive complement fixation tests were obtained in 17. These results suggest the possible value of the complement fixation test where malaria is indicated but no other positive evidence is found. A positive serological reaction may be a means of detecting malaria in cases of long standing such as is seen in the representative group studied.

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SUMARIO

Se efectuaron una serie de pruebas de fijación del complemento para malaria con suero de 174 pacientes, relacionándolas con el exámen de láminas de sangre. Entre los pacientes 145 habían tenido o habían estado expuestos a la malaria y 106 habían

servido en la zona del Sudoeste del Pacífico. Las reacciones serológicas fueron positivas en 31 de los 35 pacientes con parasitemia demostrable. Por el contrario, dichas reacciones fueron negativas en el grupo sin historia de malaria en el último año antes del examen.

De un grupo de 89 pacientes en los cuales no pudo ser demostrada parasitemia, pero con historia de malaria durante el último año antes del examen, resultaron 17 positivos a la prueba de fijación del complemento. Estos resultados sugieren la posible utilidad de la prueba mencionada en casos de malaria donde no es posible otro medio evidente de diagnóstico. Una reacción serológica positiva puede ser un medio de descubrir la malaria en casos antiguos tales como los representados por el grupo estudiado.

Resumen

Huevos de *Anopheles georgianus* King de tres hembras procedentes del Sur de Georgía son descritos y comparados con huevos de *Anopheles crucians* Wiedeman. A pesar de que se hace notar un desarrollo más extenso de la superficie dorsal del exocorion del *georgianus* esta característica no es suficiente para diferenciar los huevos entre ambas especies en todos los casos. Preséntanse dibujos de huevos de ambas especies y se dan medidas de una pequeña serie.

AN UNUSUAL WINTER POPULATION OF *ANOPHELES QUADRIMACULATUS* SAY

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This note records the presence of an unusual population of adult *Anopheles quadrimaculatus* Say at selected localities in Baker County, Georgia, during the winter 1948–49.

Various workers (e.g.: Balfour, 1928; Barber, Komp, and Hayne, 1924; Boyd, 1927; Boyd and Weathersbee, 1929; Zukel, 1949) who have studied anopheline mosquitoes in the southeastern United States in winter are in accord in reporting a great reduction in adult populations of *A. quadrimaculatus* during the colder winter months. Hinman (1934), however, reported an enormous winter aggregation of adult *A. quadrimaculatus* in an old fort more than 50 miles south of New Orleans, Louisiana. It appears that adult females gathered in the fort in November and December in numbers exceeding a million; but through January, February, and March, there was a very marked diminution in the numbers present; males were not noted in January but a few were present in February. In the southeastern United States, adult males of *A. quadrimaculatus* are generally lacking during January and February and are usually rare during December and March. Ives (1938) quoted by Hinman and Hurlbut (1940) found one adult male *A. quadrimaculatus* in a cave at Chattanooga, Tennessee, December 26, 1935, and another at Shell Mound, Mississippi, January 14, 1936. Brackin and Quinby (1939) reported a single male from southern Mississippi in a collection of February 11, 1938. The absence of adult males of *A. quadrimaculatus* during winter is so characteristic that malaria control personnel have used the appearance of the first adult male in spring as a rule-of-thumb indication of the onset of emergence of the first spring brood of the species.

The observations reported here represent weekly station examinations of artificial adult mosquito resting places in the vicinities of Mossy, Putney, and Springfield Ponds, in western Baker County, Georgia. These three ponds are characteristic of the regional Karst topography. The artificial resting places were the wooden one foot cubical red boxes described by Goodwin (1942) and U. S. Army privy-type mosquito shelters. Fifteen red boxes at the edge of Mossy Pond, eight at Putney Pond, seven at Springfield Pond, and one privy-type shelter at each of these ponds are stations routinely examined each week throughout the year as part of the Public Health Service Malaria Observation Stations program. This report treats routine observations at these mosquito resting places during December 1948, and January, February, and March, 1949. The mosquitoes observed under a worktable and about the eaves of two small instrument shelters at Mossy Pond during January, February, and March, 1949, were counted in the totals. Station examinations were usually accomplished on one day of each week, but in a few cases a second day was required to complete the examinations.

FIG. 1. Total weekly counts of adult male and female *A. quadrimaculatus* Say at mosquito resting stations near three ponds in Baker County, Georgia during the winter 1948–49. Note that below 10, the vertical scale is expanded; and above 100, is contracted.

All records are based upon recorded counts of mosquitoes at the resting places, these being examined with the aid of a flashlight; but representative specimens were often collected to support the identifications made at sight.

Figure 1 is a graph showing the total weekly counts of male and female adult *A. quadrimaculatus* at the stations in the winter 1948–49.

During December 1948, the lowest total count of *A. quadrimaculatus* males for any week was 31 (Dec. 30–31); during January 1949, four (Jan. 20); during February, 17 (Feb. 3); and in March, more than 100. The lowest weekly count of females in December 1948 was 89 (Dec. 30–31); in January 1949, 47 (Jan. 6); in February, 48 (Feb. 17); and in March, more than 100. The highest weekly count of males in December 1948, was 230 (Dec. 16–17); in January 1949, 37 (Jan. 27); in February, 231 (Feb. 24–25); and in March, more than 250. In December 1948, the highest count of females for any week was 376 (Dec. 16–17); in January 1949, 129 (Jan. 13); in February, 157 (Feb. 24–25); and nearly 200 by March 10. During the several months preceding December 1948, weekly counts of adult males of *A. quadrimaculatus* at the stations did not fall below 100 individuals and adult females regularly exceeded this figure. Similarly, the counts each week after March 10, 1949, totalled more than 100 individuals of each sex.

This unusually large and persistent population of adult *A. quadrimaculatus* of both sexes is interpreted as a natural corollary of the unusually balmy winter 1948–49. Data from the U. S. Weather Station at Albany, Georgia (U.S.W.B., 1929–1949), approximately 50 miles northeast of the study area, show that the winter 1948–49 was the warmest in the past 20 years. Considering as winter the five month period, November to March, inclusive, the winter 1948–49 had the highest mean daily temperature of a 20-year period; if March temperatures be disregarded, the winter 1931–32 was warmer. Of probable greater importance from the standpoint of mosquito survival, the number of days with freezing temperature or colder was only five during the winter 1948–49, but seven or more in every other winter of the past 20, and the mean minimum temperature for the five month period was higher in the winter 1948–49 than in any other of the past 20 winters.

Weekly collections of anopheline larvae from the ponds adjacent to the adult resting stations revealed the presence of fourth instar larvae of *A. quadrimaculatus* in at least one pond during each winter month; but some weeks, larvae of this species were not found. Although each collection consisted of only five or ten sweeping dips made with a shallow enamelware pan, the collections of larvae coupled with the counts of adult mosquitoes appear to indicate that *A. quadrimaculatus* adults emerged each month but not each week of the winter 1948–49.

Adult males and females of *Anopheles crucians* Wiedemann and *A. punctipennis* (Say) were present at the stations in varying but usually small numbers during each month of the winter 1948–49.

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Resumen

Machos y hembras adultos de *Anopheles quadrimaculatus* Say fueron observados constantemente en visitas semanales a estaciones de captura rutinarias en Baker County, Georgia.

NOTES ON THE OVA OF *ANOPHELES GEORGIANUS* KING

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During the winter and spring 1948–49 fertile ova of *Anopheles georgianus* King were obtained from at least three adult females. While the information at hand does not permit absolute differentiation between ova of *Anopheles georgianus* and ova of *Anopheles crucians* Wiedemann, there is evidence that the majority of the ova of the one species differ recognizably from the majority of the ova of the other. The ova of *Anopheles georgianus* have not been noticed previously and there is some confusion in the literature as to the ova of *Anpoheles crucians.*

Figures of the ova of *Anopheles crucians* Wiedemann, *Anopheles punctipennis* (Say), and *Anopheles quadrimaculatus* Say (as *A. maculipennis*) were given by Mitchell (1907) who also provided key characters for distinguishing the ova of these three species. In volume two of Howard, Dyar, and Knab's monographic work (1913–17) Miss Mitchell's drawings of ova were again utilized; but here, unfortunately they were incorrectly labeled, the figure of an *A. crucians* egg being labeled *A. punctipennis* and conversely the *A. punctipennis* egg being designated *A. crucians.* As subsequent authors have been disposed to follow Howard, Dyar, and Knab, the error has been perpetuated.

In our study of anophelines in south Georgia, the adult female mosquitoes were collected in the field, brought to a heated laboratory, and isolated in lantern chimney cages over finger bowls containing tap water to a depth of one to two centimeters. Bowls were examined daily for the presence of ova. Ova of *Anopheles georgianus* were obtained from two specimens collected in Baker County, Georgia, December 9, 1948, and March 10, 1949, respectively, and from a specimen collected in Thomas County, Georgia, April 9, 1949. Identity of the ova was established by rearing hatched larvae from each batch to the fourth larval instar in which stage they were identified. Figure 1 is a photomicrograph of *A. georgianus* ova from a batch of about 100 deposited during the night December 10–11, 1948, by the female collected December 9. These ova were photographed because of their dissimilarity to the ova usually obtained in this area from females of *Anopheles crucians* Wiedemann. Ova of the latter type are shown in the photomicrograph, figure 2. Typical fourth instar *A. crucians* larvae were reared from the ova shown in figure 2. These ova were deposited during the night December 12–13, 1948, and were photographed December 14, the same day that the photograph of *A. georgianus* ova (fig. 1) was taken.

As illustrated by the photomicrographs, the December batch of *A. georgianus* ova is distinctly different from the ova usually deposited by *A. crucians.* In the former, the exochorion completely covers the deck of the egg between the floats and leaves only a small slit-like area of endochorion visible at either end; while in the latter, the exochorion leaves uncovered an irregular narrow zone of endochorion centrally between the floats which is continuous with the slightly broader uncovered areas at the ends of the egg.

In our notes on anopheline ova we designate as "open" those that have a continuous zone of endochorion exposed along the deck or dorsal surface from end to end, while "closed" ova are those that have areas of exposed endochorion at each end separated by a broad or narrow isthmus of exochorion across the deck in the middle region. Thus, the *georgianus* ova shown in figure 1 are closed as are the winter type ova of *Anopheles walkeri* Theobald figured by Hurlbut (1938) and the "unusual" type ova of *Anopheles punctipennis* (Say) figured by Lawlor (1940). The *A. crucians* ova shown in figure 2 are open as are the summer eggs of *A. walkeri* figured by Hurlbut and the usual type ova of *A. punctipennis* figured by Lawlor.

FIG. 1. Ova of *Anopheles georgianus* King. The egg on the right hatched a few minutes before the photo was taken.

As indicated above, *A. crucians* ova deposited by adult females collected in southwest Georgia are generally of the type illustrated in figure 2. We have obtained batches of uniformly open ova of this type from 69 adult females. Hatchlings from these ova were pooled in rearing pans and periodic microscopic examination of fourth instar specimens developing in these pans revealed the presence of none but *A. crucians* larvae in this instar.

On the other hand, between January 1 and June 8, 1949, 24 isolated females of the *crucians* complex provided 31 batches of ova which resembled or approached those illustrated in figure 1. Fourth instar larvae were successfully reared from 21 of these batches but only two batches, those deposited by the females collected March 10 and April 9, 1949, respectively, were found to be *A. georgianus*. Identification of the fourth instar larvae reared from the other 19 batches established that they were

FIG. 2. Ova of *Anopheles crucians* Wiedemann

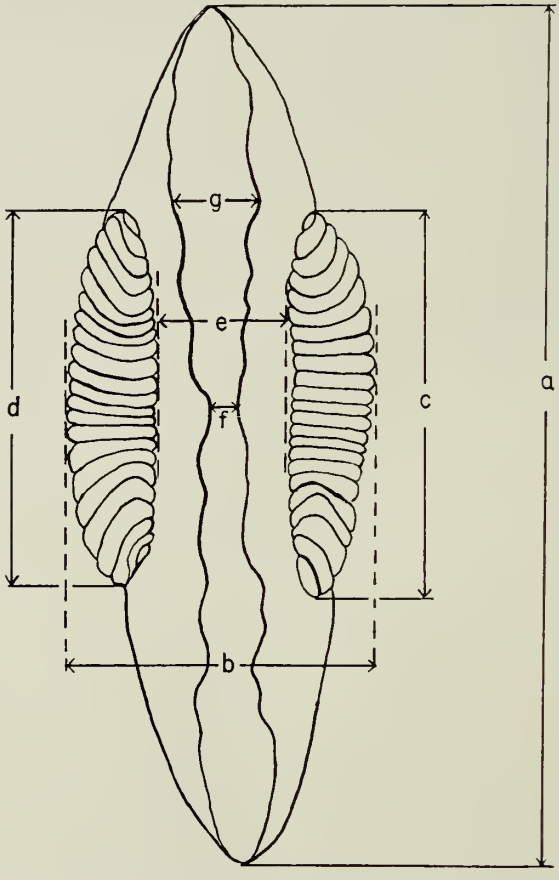

FIG. 3. Diagram of an egg of *Anopheles crucians* illustrating measurements made

A. crucians. Mortality was often high in these isolated batches of developing larvae, but in no instance did a mixture of *georgianus* and *crucians* larvae mature to the fourth larval instar from ova deposited by a single female.

There was considerable variation from batch to batch among the 31 lots of ova mentioned above. A few batches which were superficially indistinguishable from the *A. georgianus* ova shown in figure 1 were subsequently proven to be ova of *A. crucians*. In other batches the ova were only very narrowly closed, i.e. the isthmus of exochorion across the central region was very narrow. In some instances, the ova of a given batch were inconsistent, some being open and others (usually narrowly) closed.

TABLE 1

Dimensions in Microns and Number of Float Ridges of Ova of Anopheles georgianus *King and* Anopheles crucians *Wiedemann. Measurements by Ocular Micrometer to Nearest Half Scale Division (One Scale Div. = 13.8 Microns) Figures are Means with Standard Error*

EGG FEATURES	ANOPHELES GEORGIANUS 15 OVA FROM BATCH 239A	ANOPHELES CRUCIANS 15 OVA FROM BATCH 462A	ANOPHELES CRUCIANS 10 OVA FROM BATCH 469B	ANOPHELES CRUCIANS 7 OVA FROM BATCH 246A
a. Length of egg	580.57 ± 2.07	549.70 ± 2.32	509.22 ± 2.73	556.14 ± 2.38
b. Greatest breadth of egg including floats	182.16 ± 1.43	200.56 ± 1.38	184.92 ± 1.88	184.92 ± 2.58
c. Length of float ridge area on right side of egg	314.64 ± 2.32	298.08 ± 2.08	278.76 ± 3.93	306.36 ± 3.68
d. Length of float ridge area on left side of egg	317.40 ± 4.12	302.22 ± 1.62	280.14 ± 3.12	304.98 ± 4.35
e. Distance between right and left floats	72.73 ± 7.07	95.68 ± 1.11	86.25 ± 2.44	93.84 ± 3.34
f. Width of exposed endochorion at narrowest point between floats (zero in closed ova)	3.68 ± 1.57	11.04 ± 1.28	0.0	13.8 ± 0.0
g. Width of exposed endochorion at broadest point anterior to floats	41.40 ± 2.25	29.43 ± 3.25	42.09 ± 0.90	42.78 ± 1.84
Number of float ridges, right side	21.40 ± 0.31	22.67 ± 0.24	21.22 ± 0.47	23.86 ± 0.47
Number of float ridges, left side	22.53 ± 0.34	22.93 ± 0.32	21.77 ± 0.34	23.71 ± 0.33

The *georgianus* ova provided by the Baker County female collected in March were only narrowly closed and the specimen of *georgianus* collected in Thomas County in April deposited a mixed batch of ova, the majority closed but some being open. This female took blood following oviposition and provided a second batch of ova. All of the ova of this second batch were open; these ova hatched, but the larvae were not examined as no individual attained the fourth larval instar. Thus the *georgianus* ova obtained from the two females collected in the spring 1949 were superficially less similar to the first batch of *georgianus* ova than were the few broadly closed lots of *crucians* ova mentioned above.

It is apparent that *crucians* ova are generally open and that *georgianus* ova tend to be closed, but *A. crucians* occasionally deposits closed ova and *A. georgianus* may deposit open ova. Also both species on occasion deposit mixed batches.

The ova of the two species were compared as to their dimensions and the number of float ridges. As unhatched, viable ova are much more satisfactorily measured than are hatched or preserved ova, measurements of the former only are given. Our inability to distinguish between ova of *georgianus* and *crucians* prior to rearing complicated the selection of representative viable ova for measurement. All measurements were made by ocular micrometer. On the dorsal aspect of each egg the following dimensions were measured as diagrammatically illustrated in figure 3, and float ridges on the right and left side were counted:

a. length of egg
b. greatest breadth of egg including floats
c. length of float ridge area on right side of egg
d. length of float ridge area on left side of egg
e. distance between right and left floats
f. width of exposed endochorion at narrowest point between floats (zero in closed ova)
g. width of exposed endochorion at broadest point anterior to floats

As we are unable to find on record any measurements of the ova of these species, the results of our measurements are presented (table 1), but the series measured is small and the measurements do not provide reliable criteria for distinguishing the ova of *A. crucians* from those of *A. georgianus*. The magnitude of the differences between batches of *crucians* ova deposited by different females may be noted.

DISCUSSION

In spite of the morphological similarity of adults and ova of *Anopheles crucians* Wiedemann and *Anopheles georgianus* King no evidence of intergradation was obtained in this study. On the contrary, the fact that the fourth instar larvae reared from the ova deposited by any female were without exception of one species lends support to the concept that the forms are biologically distinct and thus separate species (King and Bradley, 1941). Vargas (1941) in Mexico, has provided notes on the ova of *Anopheles bradleyi* King, the third member of the *crucians* complex.

Cross-breeding and physiological testing along the lines of Farid's work (1949) with *Culex pipiens* L. and *Culex quinquefasciatus* Say greatly improves our understanding of the relationship of populations of mosquitoes. Colonization of such closely related forms as *A. crucians*, *A. georgianus*, and *A. bradleyi* is an urgent prerequisite to such experimental work.

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A METHOD OF EVALUATING DENSITY OF ANOPHELINE BREEDING FOR PURPOSES OF MALARIA CONTROL

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During 1944 and 1945 the writer was confronted with the problem of controlling malaria among United States Navy and Chinese personnel in widely separated areas in China. An important part of this malaria program consisted of mosquito control.

The terrain in areas under consideration was mountainous or hilly. The areas were primarily agricultural, the staple crop being rice grown in flooded paddies. The rice paddies varied in size from a few square feet to an acre or more, and were ideally adapted to the production of anopheline mosquitoes. The only anopheline species universally distributed and occurring in numbers sufficient to be a malaria hazard was *Anopheles hyrcanus* var. *sinensis* Wiedemann.

In 1944 the first anopheline larvae were found in March, and they continued to be found in uncontrolled areas as late as December of that year and as early as January 1945. It is likely that breeding continued throughout the year in warm southern areas of low altitudes, and that in some regions mosquitoes passed the winter in the larval stage. New malaria cases failed to occur in significant numbers during the winter months.

The unavailability of the usual larvicides in China necessitated study of readily available oils and their adaptation to the destruction of the immature forms of anopheline mosquitoes. In the absence of diesel and other mineral oil products in China the Chinese developed a method of recovering a substitute diesel fuel from tung oil. Since tung diesel fuel was available for the operation of trucks and electric generators it was decided to experiment with this material as a larvicide. Results of these experiments were most encouraging and the use of this material became routine wherever it was available.

In order to organize intelligently a larvicide schedule with as little overhead cost as possible in manpower or in critical materials, and to evaluate the effectiveness of the locally developed insecticide mixtures quantitative data had to be collected. An inspection form was developed, designed to show at a glance the anopheline breeding situation, the results of previous applications of larvicides, and to forecast the date when subsequent treatment would be indicated. Previously developed report forms and methods were lacking in certain essential features. Methods described by Boyd (1930) were among the first attempts to apply quantitative procedures for evaluating density of anopheline larvae in natural breeding places. Cambournac (1939) in Portugal has described methods for determining larval population in rice fields. Goodwin and Eyles (1942), Hess (1941), and Hess and Hall (1943) described methods used for determining larval concentrations in limited areas for experimental purposes, in

* The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

the United States. Russell, Rao, and Putnam (1945), and Russell and Rao (1941) described methods used to determine larval density in experiments on seasonal prevalence of *Anopheles* in India. The report form described in this paper and the method recommended for its use is solely an attempt to revise and improve inspection procedures in the interest of economy, administration, and efficiency. As a rule methods and report forms dealing with the density of anopheline larvae have been designed to study biological conditions rather than to assist in the practical administration of malaria mosquito control. None has been completely satisfactory in presenting quantitative data pertaining specifically to practical control problems. Of all methods so far described to discover and evaluate density of anopheline larvae for malaria control purposes the report form suggested by Bradley (1943) most nearly fitted the requirements for a larval inspection report form. Unfortunately the writer did not see Bradley's publication until 1948.

Innumerable attempts have been made by investigators and by practical malaria control operators to "standardize" the inspection dip. Some of these attempts have been quite satisfactory for specific problems of research, but have not lent themselves to general application in control programs. Boyd (1930) advocates the use of a dipper specially constructed so that a more or less definite area of water surface is covered by a sweeping type dip. This method has several disadvantages from the standpoint of bulk and unwieldy manipulation, and from the fact that in nature anopheline larvae usually are found not evenly distributed over a water surface so as to permit a representative sweeping dip. Nevertheless this method has been used widely particularly in experimental work where it was important to have a record of actual numbers of anopheline larvae encountered. Similarly Hess (1941) for purposes of investigation designed a special dipper for sampling measured water surface areas. This design is admirably suited for its purpose but the procedure is too unwieldy for practical control inspection. The same criticism can be applied to the devices and methods used by Goodwin and Eyles (1942) which are adequate and ingenious but do not meet the problem posed by the malaria control operator who wants to know certain information quickly about a large series of breeding areas. The information needed is: (a) whether anopheline larvae are present, (b) if so, when it is necessary to institute control procedures, and (c) what type of control procedures are indicated.

Doctor T. H. D. Griffitts (1937) often stated that Doctor Henry R. Carter, "grand old man" of malaria control, classified dips as "suction dips, sweeping dips, and combination sweeping and suction dips". The inference is that in order to discover the presence of anopheline larvae with reasonable surety the inspector should determine from the local conditions just what kind of a dip he would have to make and where, but the underlying objective is to find the larvae if any are present. That is the purpose of the inspector. It is relatively unimportant to know how many larvae occur per square meter. For purposes of control if any are present they should be eliminated.

When assigned the task to discover the presence of anopheline larvae in an area subject to malaria control operations, it becomes relatively unimportant just what type of dip or dipper is used. Individual inspectors have their preferences and feel more confident in the use of the procedures which they have learned by experience suits them best. As a rule the writer does not approve of any dipper or dipping

method permitting the addition of an extension handle since the inspectors invariably are too far removed from the tiny organisms for which they are searching.

The generalization concerning standardization of the dip does not apply to the larval inspection report form. It must be standardized in order to organize and to operate efficiently a malaria mosquito control program. As with other operations in the field the simpler the form, the better, provided it is consistent with the data needed.

A reproduction of the inspection form herein described, obverse and reverse, follows:

MALARIA CONTROL

Dipping Report

Date---------- Insp.----------

No.	+	++	+++	++ ++	IV-P	No. Dips	No. Pos.	Cul. √ or-	Re-marks

Obverse

\+ 1–5 Larvae per dip, stages I–III, inc
\+ + 6–10 " " " " " "
\+ + + 11–20 " " " " " "
\+ + + + over 20 " " " " " "

Report IV & P by numbers separately

Reverse

FIG. 1. LARVAL INSPECTION FORM.

Obverse. First eight columns from left refer only to anopheline larvae. "IV" refers to fourth stage anopheline larvae. "P" refers to anopheline pupae. "√" in

column nine indicates the presence of culicine larvae; "—" indicates no culicine larvae were found.

Reverse. Refers only to anopheline larvae.

This form was printed on 3 x 5 cards. The blank space on the reverse was used for sketches, when necessary, to identify breeding places or for additional notes. Card data were summarized for routine monthly reports. A study of completed forms following inspection provided the following important information:

(1) An estimate of the amount of anopheline breeding in the area.
(2) An accurate check on the stage of growth of aquatic forms of anophelines in the area.
(3) Evidence determining the most economical time at which to apply larvicides.
(4) A check on the success of the previous mosquito control operations carried out in the area.
(5) A comparison (if desired) of one area with another in order to evaluate the efficiency of one method with another, or the efficiency of one control team with another.

All series of dips were comparable whether or not the size of the dipper used was standard in each series. In practice the bottom half of standard military mess gear was used as a dipper. This gear was always available but seldom if ever used for its designed purpose among Naval Group China personnel, since these personnel lived and subsisted in the Chinese manner. The actual dip-sites in each inspection were selected by the Pharmacist Mate or by the Malaria Control Officer making the inspection from the places which in their experience were most likely to be harboring larvae and pupae. No attempt was made to correlate the number of larvae and pupae with the area of the breeding place, or to make a dip covering any measured surface area. (It is well known that anopheline larvae tend to congregate in, or at the edge of, masses of floatage, around the stems of emergent vegetation, and in such other places where the micro-environment is suited to their needs. They are seldom if ever uniformly distributed over the water surface). The purpose of inspection is to discover the presence of any anopheline breeding, hence concentration on the most likely places harboring aquatic stages.

In explanation of the inspection report form, it may be stated that it is desirable but not essential in a continued program for malaria mosquito control to prepare a map with all known breeding places designated by number or symbol and to record such designations in the first (left hand) column of the form. In practice this desirable but time-consuming refinement was sometimes omitted. If breeding places are so identified it often will be found that certain breeding places show fourth stage larvae, and pupae, more frequently than others. This may be due to characteristics of the terrain making it difficult or unpleasant for larvicide crews to treat effectively these areas, or to individual properties of the breeding place more conducive to anopheline development. Such places may require special attention.

Reading from left to right the second column lists one-plus (+) dips. In this column are recorded those dips which are "positive" for between one and five (inclusive) anopheline larvae of stages I, II, or III. In the third column two plus (++) dips are listed. These are dips which are "positive" for between six and ten first,

second, or third stage larvae. Three plus (+++) dips and four plus (++++) dips record eleven to twenty and over twenty such larvae per dip in the fourth and fifth columns, respectively.

The column headed "IV" and "P" is used to record the actual number of fourth stage anopheline larvae and pupae found in the dips in each breeding place. If none of either are found it is obvious that control operations are not needed immediately. If anopheline pupae are found in large numbers in all breeding places a contact larvicide such as oil, or oil and DDT, is indicated immediately. If no pupae, or merely an occasional one is found, but many fourth stage larvae are present, larvicide operations using contact insecticides are indicated but may be postponed one to three days. If most breeding places inspected show only very young anopheline larvae (stages I and II) but an occasional one shows IV and/or P this indicates that either (a) the breeding place was not adequately treated during the previous larvicide operations if any, or (b) older developmental stages of anophelines were introduced into the breeding place by drainage into the area or by some other means. Reconnaissance may determine whether one of the variables may be eliminated. These data show the amount of anopheline breeding in the area, and more important, the stage of development of the anophelines.

The presence or absence of culicine species was simply noted in the appropriate column, as (√) or (−). This information relative to culicines was of little practical value, though results did indicate that culicines were more resistant to DDT treatments than were anophelines.

In the column headed "Remarks" the inspector may make a note, such as "all I" or "mostly II", or "I, II, III", to indicate roughly the stage of development among anopheline larvae found. This assists the officer in charge to forecast when the next control operation must be planned.

In practice it was found desirable to select wisely certain well distributed breeding areas apparently preferred by adult anopheline mosquitoes for oviposition, and to repeat inspections in these areas since by experience they more often showed breeding than others, or routinely showed more breeding. In addition other areas were selected haphazardly or at random in order that undue weight might not be placed on an uncertain variable. It was impractical, if not physically impossible, to inspect all breeding places routinely.

In the hands of the writer and his assistants in widely separated areas in China the described inspection report form for evaluating the density and distribution of anopheline breeding in a malaria control program proved superior to published methods both for routine use and for research on previously untried larvicides. In addition, use of the inspection form indicated the dates when the most economical application of larvicides should be effected as well as the type of larvicide to be used (whether stomach or contact poison).

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THE RELATIVE EFFECTIVENESS OF DDT AND DDD AS ANOPHELINE MOSQUITO LARVICIDES UNDER FIELD CONDITIONS

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It has been demonstrated that DDT (2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane) is a satisfactory larvicide for the control of anopheline mosquito larvae when applied as an oil-mist spray at very low dosages (Ferguson, Arnold and Upholt, 1947, Mathis, Ferguson, Simmons 1947). It is, however, also toxic to a certain degree to other aquatic organisms. This is especially true when used at weekly intervals throughout a breeding season as is done in most control work (Tarzwell 1948). Preliminary field work in 1946 indicated that DDD (2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane) is also an effective anopheline larvicide. Moreover, DDD is indicated to be less toxic to other aquatic organisms, especially fish (Tarzwell 1948).

To determine if DDD could be substituted for DDT as an anopheline larvicide, comparative larvicidal tests were made in 1947 with various dosages of DDT and DDD. Almost all of the work was done in the Camp Stewart Military Reservation located near Savannah, Ga. The test plots were selected from natural land-locked bodies of water which contained suitable numbers of anopheline mosquito larvae. The ponds treated varied greatly, ranging in size from 2,400 to 44,000 square feet and in vegetative cover from open ponds with only marginal vegetation to densely covered ponds. Sampling was done by means of long-handled dippers four inches in diameter and the larval instars were determined in the field. The studies entailed pretreatment sampling, followed by one- and three-day post-treatment larval counts with the lower dosages and an additional seven-day larval count with most of the tests at the higher dosages. Gross observations were made on the condition of other aquatic organisms at each visit to an experimental plot. Untreated check ponds were sampled from time to time to assure that weather and other environmental conditions remained suitable for mosquito breeding. No direct comparison was made between treated and check ponds because of the extreme variability from pond to pond.

Fuel oil No. 2 was selected as the solvent, since former work had indicated it to be satisfactory with reference to its dissolving capacity for either material, cost, availability, and larviciding results obtained (Ferguson, Arnold and Upholt 1947, Mathis, Ferguson and Simmons 1947). Previous recommendations from this laboratory included 0.5 per cent of a spreading agent in the larviciding formula and Triton B-1956[2] was the agent chosen for this (Mathis, Ferguson and Simmons 1947).

[1] From Technical Development Division, Savannah, Ga.

[2] Product of Rohm and Haas Co., Philadelphia, Pa. The trade names are carried as a means of dentifying products under discussion, and do not represent endorsement of the products by the Public Health Service.

EQUIPMENT AND OPERATIONS

The equipment used in this work consisted of a one and one-half gallon Hudson Clipper sprayer equipped with a four-foot oil-resistant hose and a wand two feet in length. The nozzle used was a Monarch 5.00. The sprayer was operated at a pressure of 30–40 psi, at which the discharge rate was about three gallons per hour. A pressure gauge was installed on the sprayer so as to observe the operating pressure, and a valve was provided to release the pressure before removing the pump to refill.

In each experimental plot, a sufficient number of dips was made to obtain an adequate sample of the pretreatment larval population, and if the average was one or more larvae per dip, it was considered satisfactory for treatment. The size of the plot was estimated and sufficient material placed in the sprayer to cover the plot. The sprayer was then pumped to 40 pounds pressure and not allowed to fall below 30 pounds during the spraying operation. In treating the plots, the operator moved at a slow pace (approximately 75 feet per minute) through the area at 30-foot intervals until the area was covered. The vaporous oil mist discharged by the nozzle was windborne, and a swath width of 30 feet was used since previous work indicated that satisfactory results could be obtained under most conditions with this width (Ferguson, Arnold and Upholt 1947, Mathis, Ferguson and Simmons 1947). The wind velocity determined the height at which the nozzle was held. With a wind velocity of two to three m.p.h. the nozzle was held about shoulder high, and as the wind velocity increased, the nozzle was lowered so that most of the spray would fall within the 30-foot swath. It was necessary to exercise extreme caution as the wind velocity approached 10 m.p.h. in order to prevent the spray from being carried over the 30-foot swath. Care was also exercised in mixing the larvicide and filling the sprayer in order to keep the solution and sprayer clean to prevent clogging of the nozzle.

EXPERIMENTAL FIELD RESULTS

Limited comparisons were made during 1946 with DDT and DDD at dosages ranging from 0.001 to 0.05 pound per acre.

The two lower dosages gave such erratic results that comparative evaluations are of doubtful value. The reductions obtained with either material at dosages of 0.05 or 0.025 pound per acre were quite similar for the first five days. Inasmuch as no evidence has been obtained that the low dosages give any residual effects, the variations after the five-day period probably reflect the high degree of variation normally present in check ponds rather than any results of the treatment.

In the course of the studies performed during 1947, applications of each material were made at the rate of one gallon of solvent per acre. The amount of DDT and DDD was varied so as to produce final applications ranging from approximately 0.0125 to 0.05 pound per acre. Previous work with DDT applied at the rate of 0.05 pound per acre gave better than 90 per cent control (Ferguson et al. 1947). Since under field conditions it is often difficult to distinguish between the effectiveness of two materials when the reduction is this high, the two lower dosage rates were included for comparative purposes, and not with the expectation of securing adequate larval control. An attempt was made to make the applications of the two materials as comparable as

possible. Approximately the same number of treatments were made each day for the two materials and in as similar types of ponds as possible. Table 1 gives the results obtained with each material when applied at rates varying from 0.0125 to 0.05 pound per acre in one gallon of No. 2 fuel oil.

The larval mortality for each material was computed from the averages of the combined number of dips and larvae found in the pre- and posttreatment examinations of all plots. Student's t-test after angular transformation indicated no significant differences in the effectiveness of the two materials.

With dosages below 0.05 pound per acre of either material, the results were so erratic that it was deemed unwise to make any comparative evaluations. It does give additional evidence, however, that 0.05 pound per acre is the minimum dosage at

TABLE 1

Relative Effectiveness of DDT and DDD at Various Dosages for Control of Anopheline Larvae—1947

RATE OF APPLICATION PER ACRE	NUMBER OF REPLICATE TESTS	AVERAGE PER CENT LARVAL MORTALITY		
		1 day	3 days	7 days
DDT 0.0125 lb., Fuel Oil, 1 gal.	9	61	40	—
DDD 0.0125 lb., Fuel Oil, 1 gal.	11	67	60	—
DDT 0.025 lb., Fuel Oil, 1 gal.	9	56	59	—
DDD 0.025 lb., Fuel Oil, 1 gal.	8	72	62	—
DDT 0.05 lb., Fuel Oil, 1 gal.	19	89*	86	35
DDD 0.05 lb., Fuel Oil, 1 gal.	17	95†	83	33

* Based on 9 tests. This figure is a little lower than expected on the basis of experience in other experiments before and after this one.

† Based on 7 tests.

which adequate control can be secured consistently with the present solvent and method of application.

The amount of emergent vegetation in the treated plots apparently did not greatly affect the larval mortality. The lowest mortality in tests with both materials was in comparatively open ponds that had rather compact tufts of grass in them. Nothing was noted that would indicate any residual effect of either material as reinfestation was occurring by the third day in many plots, and on the weekly check some plots had a population greater than the original pretreatment population.

SUMMARY

In replicated tests under natural field conditions, no significant difference could be detected between the effectiveness of DDT and DDD as anopheline mosquito larvicides, when both materials were applied at the rate of 0.05 pound per acre in one gallon of No. 2 fuel oil with 0.5 per cent spreading agent added.

Such erratic results were obtained with dosages below the 0.05 pound per acre level as to make valid comparisons impossible. These results confirm previous findings that 0.05 pound per acre represents the minimum dosage level at which DDT

(and DDD) may be applied by hand sprayers with present methods, materials, and equipment in order to obtain satisfactory anopheline larval control.

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SUMARIO

No pudo descubrirse ninguna diferencia significante entre la efectividad del DDT y del DDD como larvicida contra mosquitos con aplicación de ambos materiales a la rata de 0.05 lbs. por acre en un galón de fuel oíl N° 2 with 0.5 por ciento de un agente de dispersión.

Estos resultados erráticos se obtuvieron con dosis inferiores a 0.05 lbs. por acre lo que hizo imposible comparaciones que tuvieran algún valor. Estos resultados confirman hallazgos anteriores de que 0.05 lbs. por acre representa la mínima dosificación a la cual DDT y DDD puede ser aplicado con bombas rociadoras de mano con los métodos materiales y equipos disponibles al presente para obtener un control satisfactorio de larvas anophelinas.

THE LIBERIAN INSTITUTE TAKES SHAPE

Early in 1946, The American Foundation for Tropical Medicine undertook to establish in Liberia, West Africa, a research center for a broad attack upon the diseases of the tropics. Little did its Directors realize at that time that nearly four years would be required to bring their plans to fruition. Unforeseen difficulties of one kind and another have blocked the start of construction and delayed the development of extensive plans but at long last the building program is under way. The first phase of construction calls for the completion of three 2-bedroom houses and Section 1 of the laboratory.

Many have inquired from time to time, why Liberia? And where in Liberia is the Institute to be located? What are to be its aims and objectives? Are the facilities of the Institute to be open to all qualified and properly accredited scientists who desire to do research or pursue advance studies there?

It may be recalled that in 1941 and extending into 1945, The American Foundation for Tropical Medicine in cooperation with Harvard Medical School and with the underwriting of the Firestone Plantations Company sent a research team into Liberia for an exhaustive study of African trypanosomiasis. This team made up of Drs. Bequaert, Veatch and Weinman investigated various phases of that vexing disease and prepared a report which was later published as a Supplement to the Journal of Tropical Medicine (September, 1946)[1]. So apparently pleased were the Firestone interests with the results of this study that the following year a gift of $250,000 was offered The American Foundation for Tropical Medicine to establish a permanent research base in Liberia, with the understanding that the Foundation would assume the responsibilities of financing the annual operating expenses. Other stipulations called for the designation of the Institute as a memorial to the late Mr. Harvey S. Firestone, that a Scientific Advisory Committee be formed made up of representatives of at least ten leading university medical schools, that there be no restrictions as to race, creed or color, and that all information and knowledge resulting from the operation of the Institute be disseminated freely.

One of the early situations facing the American Foundation was the problem of meeting annual operating expenses. This required a position of priority on Foundation funds in favor of the Liberian Institute. Our Board felt at that time and continues to believe that the establishment of such a foreign research depot is an important addition to the educational and research facilities of the United States justifying first call on Foundation funds. A meeting with representative drug and pharmaceutical houses in the Spring of 1946 resolved this point of view and the Foundation decided to accept the gift and go ahead with the Institute.

A liberal working agreement with the Liberian Government has since been negotiated, granting the Institute in perpetuity a site of 100 acres in the vicinity of Roberts Field, international airport for West Africa. This site, together with an additional 600 acres which is available when the Institute is ready for a research program in veterinary medicine and agricultural nutritional studies, will constitute

[1] Human Trypanosomiasis and Tsetse-Flies in Liberia—Vol. 26, No. 5.

the base of operations. This agreement provides among other stipulations that the Institute must conduct its operations without discrimination as to race, nationality, creed or color; that research in tropical and geographical medicine, public health and the prevention of human and animal diseases in Liberia shall be carried on and information furnished the Liberian Government; that no taxes of any kind shall be imposed upon the property of the Institute, its buildings, equipment and supplies; likewise there shall be no taxes on salaries or other funds provided Institute personnel, whether permanent or temporary.

Their Government has granted a duty free status on all equipment, supplies and materials imported into Liberia as well as on all exports of medical specimens, records, data and other materials relating to the activities of the Institute. Accredited personnel of the Institute may engage in any research projects whatever including the practice of veterinary medicine without license by the Government. They may further establish and maintain a state of quarantine in such areas as are desired for controlled studies. The Government will assist such personnel in obtaining access to and egress from all sections of the country in order to set up field stations. In short, the Institute and its personnel may carry on as broad and intensive a program as it desires with the full expectation of cooperation on the part of the Government.

For those who know little of Liberia or have limited comprehension of its location and terrain, a brief description of the country may be helpful. According to Dr. Joseph C. Bequaert, the country has an extimated area of some 43,000 square miles, an Atlantic coastline of 350 miles running northwest southeast and lies in the bulge north of the Ivory Coast in latitude 4° to 7° north. In its hinterland it reaches back some 150 miles from the coast and is roughly the size of the state of Ohio. Its population is just under 2,000,000 made up of indigenous tribes scattered throughout the country.

The coastal area for some 20 miles inland is generally flat and cut up by tidal lagoons and creeks into which rivers empty, their mouths usually being obstructed by sand bars. There are a few hilly promontories along the coastal plains, the conspicuous ones being Cape Mount (1,100′), Cape Mesurado at Monrovia, Baffu Point and Cape Palmas. Beyond the narrow coastal strip, gently sloping hills of 300 to 900 feet cover most of the country. In only a few areas do these hills rise higher, giving the appearance of mountains as in the northwest where they rise 1,400 to 2,200 feet. To the south the most conspicuous range is near the native town of Pandamai and has an elevation of 4,528 feet. It is known as the Walo Mountain or the Wolagwissi Mountain and is covered with the same rain forest heavy growth as are the surrounding lowlands. Another important range is the Nimba Mountain with altitudes of 6,000 feet or more and lies in the north central corner near the French Guinea border. Its highest elevation is reached in French territory and its entire sides with the exception of the summit are covered with rain forests. Numerous rivers and streams drain the country of its abundant rainfall. None is particularly large, many being only a few hundred yards wide. A few rivers, the Mano or Gbea River, the Laffa River, the St. Paul River, the St. John's River, and the Cavalla River help form the country's natural boundaries. Most of the rivers, including the larger ones, are shallow, swift flowing and obstructed by rocks, rapids and falls which make navi-

gation for commercial purposes impractical. In addition, the water level fluctuates greatly with the wet and dry seasons, resulting in either practically dry river beds or rushing torrents.

The climate of the country is typically tropical with a uniformly high temperature, heavy rainfall and high humidity. The temperature ranges from 60° to 90° with an average yearly mean temperature in the shade of 78.2° F at Harbel. Temperatures remain about the same along coastal areas year in and out. On traveling inland, however, temperatures drop somewhat and it is cooler throughout the year in the hilly sections with greater seasonal fluctuation. The rainfall is extremely heavy, Liberia having one of the heaviest rainfalls of any section of Africa. Along the coastal belt it rains throughout the year with the heaviest rains in the May to October period. Over an eleven year cycle at the Firestone Plantations headquarters at Harbel (1932–42) the annual rainfall averaged 138.04 inches with a maximum of 188.85 inches in 1933. January and February are usually the two driest months. Relative humidity in the coastal area remains high practically the year around, 98 to 100 per cent at night and somewhat less during the day. During the "wet" season it falls off less while in the period December to April it may fall below 75 per cent. Such high humidity accounts for the very heavy mists which often blanket the land in the morning hours even in the "dry" season. In the hinterland, the humidity tends to become lower. Thus the climate and rainfall present ideal conditions for tropical rain forest growth which spreads well over the entire country.

An understanding of the terrain, climate and rainfall conditions of the country is most helpful in appreciating the ideal area in which the Institute is being located for broad research in tropical diseases. It has been said that most every type of tropical disease can be found in Liberia. In addition with the country's being somewhat small in area and stretching from the seacoast to the high hinterland, research of many types is practical without having to range too widely.

It is the intent of the Board of the Institute to make it an international center for research. Its program will be screened by a Scientific Advisory Committee made up of representation from 29 leading medical schools, governmental departments and commercial organizations as follows:

Bowman Gray School of Medicine, Winston-Salem, S. C.
Columbia University School of Public Health, New York, N. Y.
Duke University School of Medicine, Durham, N. C.
Harvard University Medical School, Boston, Mass.
Harvard University School of Public Health, Boston, Mass.
Howard University School of Medicine, Boston, Mass.
Johns Hopkins University School of Medicine, Washington, D. C.
Long Island College of Medicine, Brooklyn, New York
Meharry Medical College, Nashville, Tenn.
New York University College of Medicine, New York, N. Y.
The Ohio State University, Columbus, Ohio
Stanford University School of Medicine, San Francisco, Calif.
Tulane University of Louisiana School of Medicine, New Orleans, La.
University of California Medical School, San Francisco, Calif.
University of Chicago, School of Medicine, Chicago, Ill.
University of Michigan Medical School, Ann Arbor, Mich.

University of Oklahoma, Norman, Okla.
University of Pennsylvania School of Medicine, Philadelphia, Pa.
University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pa.
University of Southern California, Los Angeles, Calif.
College of Medical Evangelists, Loma Linda, Calif.

American Cyanamid Co. Research Laboratories
Winthrop-Stearns, Inc.
Eli Lilly & Co. Research Laboratories
United Fruit Company

Army Medical College
Gorgas Memorial Institute, Panama
Bureau of Medicine & Surgery, U. S. Navy, Washington, D. C.
U. S. Public Health Service, National Institutes of Health, Bethesda, Md.

In addition a group of leading foreign schools will be asked to name representatives to a Board of International Consultants which will advise on fields of research in other foreign lands. It is planned to make the facilities of the Liberian Institute available to their scientists in return for exchange use of their foreign research establishments where a particular problem can be more adequately studied in another area. In this way a properly accredited scientist may be able to go to any tropical section of the world for optimum research conditions in the diseases of his choice. It is hoped thereby to create a better basis of international relations through the mediums of tropical medicine. Such institutions as the London School, the Liverpool School, the Calcutta School, and the Pasteur Institute have been asked to name foreign consultants.

In signalizing the start of the Liberian Institute in 1946, Dr. Thomas T. Mackie, its president, made the observation that . . . "such a partnership between Government, Business and Science presents both great opportunity and a challenge. The Foundation recognizes the responsibility which lies before it and the unique opportunity which its acceptance implies. . . . Its objectives shall be: to conduct a direct attack on a broad scale against the several segments of the vicious circle of endemic medical problems in the tropics; to aid in the development and improvement of local economies for the benefit of indigenous populations and for business both local and foreign; to provide stimulus and facilities for educational institutions of the United States for research and teaching of better international understanding." These objectives have remained unchanged and have in fact been implemented in the past four years. It is expected the Institute will be ready for operation late in 1950.

32ND ANNUAL MEETING OF THE NATIONAL MALARIA SOCIETY

Held Conjointly with the American Society of Tropical Medicine and the American Academy of Tropical Medicine

MINUTES—1949

Officers

President—Dr. Wendell Gingrich, Galveston, Texas
President-Elect—Dr. Paul F. Russell, New York, New York
Vice President—Dr. Ernest Carroll Faust, New Orleans, La.
Secretary-Treasurer—Dr. Martin D. Young, Columbia, S.C.

Directors:

Mr. W. H. W. Komp, College Park, Maryland
Dr. E. L. Bishop, Chattanooga, Tennessee
Mr. H. W. Van Hovenberg, Mt. Pleasant, Texas

Monday, November 7, 1949

The National Malaria Society convened for its 32nd annual meeting in the Hotel Peabody, Memphis, Tennessee, at 9:17 a.m. with President Wendell Gingrich presiding. Doctor Gingrich announced the election of the following new officers for 1950:

President—Dr. Paul F. Russell
President-Elect—Dr. Justin M. Andrews
Vice President—Mr. W. H. W. Komp
Director (for 3-year term)—Dr. Lloyd E. Rozeboom

At the conclusion of the President's Address, eleven papers were read and one presented by title. During the meeting, distinguished foreign guests were introduced. The session adjourned at 12:30 p.m.

A hospitality session was held from 5:00–7:00 p.m.

Tuesday, November 8, 1949

The Society reconvened at 9:00 a.m. for a conjoint meeting with the American Society of Tropical Medicine during which the Presidents of both societies presided. A program of ten papers was presented. The meeting adjourned at 11:45 a.m.

A hospitality session was held from 5:00–7:00 p.m.

Wednesday, November 9, 1949

The scientific session reconvened at 9:25 a.m. Eleven papers were read and two presented by title. Doctor Gingrich introduced the incoming President, Dr. Paul F. Russell, who announced the appointment of Dr. Harry Most to the Editorial Board

for a 3-year term and of Dr. Lowell T. Coggeshall to represent the Society in investigating the question of the distribution of *Plasmodium berghei* in the United States. The meeting was adjourned *sine die* at 11:55 a.m.

The business meeting was held at 8:50 a.m. with President Gingrich presiding. The minutes of the 1948 annual meeting in New Orleans, Louisiana, were approved as published in the March, 1949 issue of the *Journal of the National Malaria Society.*

The Secretary-Treasurer reported as follows:

From the 1948 roster of 572 active members, 2 (Mr. C. C. Kiker and Dr. Lucien Van Hoof) have been lost by death; 17 have resigned; and 40 have been dropped because of delinquency in dues. During the year 54 new members were elected, making an active membership of 567 and representing a loss of 5 members. Of these, 451 were in good standing on October 31, 1949.

The status of the treasury at the close of business on October 31, 1949, was:

Balance reported November 30, 1948	$6,137.86
Receipts from delinquent, current, and advance dues, subscriptions, advertising, sales of back issues, interest, et cetera	4,011.41
Total	$10,149.27
Less Expenditures before paying for the 4th issue of the 1949 Journal, but including the cost of printing the 4th number of the 1948 Journal	4,566.53
Balance on Hand	$5,582.74

Of the balance on hand, October 31, 1949, $5,167.85 is in the publication account, and $414.89 is in the operating account.

Assets estimated for the year 1949, ending December 31, including the above cash balance, total $6,282.74; estimated liabilities are $1,177.50 which leave the estimated net resources available at the end of the year to be $5,105.24.

This report was accepted by the Society.

Dr. George H. Bradley, Chairman of the Committee on Auditing, stated that the books and accounts of the Secretary-Treasurer had been examined and found in order; he noted that the work of the Committee was greatly facilitated by the audit and report prepared by a Certified Public Accountant and recommended that this practice be continued in the future. The Committee also recommended that honoraria be granted in the amount of $200.00 to the Secretary's stenographer and $25.00 to the Editor's stenographer. The Society adopted the report and recommendations of the Committee.

The report of the Board of Directors' meeting November 6, 1949, was read and accepted.[1]

In the absence of Dr. A. D. Hess, the Chairman of the Committee on Advertising, the Secretary read the report which stated new advertisers had been secured by direct, personal contacts with individuals in industry. The members were urged to cooperate in trying to get additional contracts for advertising. The report was adopted.

Dr. E. C. Faust presented a short report for the Committee on Statistics, of which he served as Chairman, and this was accepted.

[1] The minutes of this meeting are printed elsewhere in the Journal.

Reporting for the Committee on Policy of which he acted as Chairman, Dr. P. F. Russell said that since the sentiment of the members remained similar to that expressed last year, no immediate changes in the Society's activities were contemplated. He recommended that the Committee be discontinued temporarily. Both the report and the recommendation were passed.

Dr. C. G. Huff, Chairman, read the report of the Committee on Lectureship and Award which was subsequently adopted.

For the Committee on Resolutions, Dr. E. H. Hinman introduced resolutions expressing the Society's regret upon the death of Mr. C. C. Kiker and Dr. Lucien Van Hoof, instructing the Secretary to transmit to each of the bereaved families an appropriate expression of sympathy. Other resolutions conveyed the appreciation of the Society to the Committee on Local Arrangements; to the Committee in Charge of Arrangements for Ladies' Entertainment; to the University of Tennessee Medical School and the Memphis and Shelby County Medical Society, the host organizations for their generous efforts which contributed greatly in making the meetings a success; and to the management of the Hotel Peabody for the adequate facilities provided. The National Malaria Society adopted all of the proposed resolutions.

After a discussion of the election procedures, the Society voted to continue the practice of sending nomination blanks in 1950 before the official ballot is prepared and mailed.

The Society favored the indorsement of the nomination of Dr. William Crawford Gorgas for election in 1950 to the New York University Hall of Fame for Great Americans.

Dr. E. H. Hinman moved that the Society indorse the recommendation of the Board of Directors that the incoming President appoint a committee to draft criteria to be used in determining the time when malaria ceases to be an endemic disease in the United States. An amendment was offered by Dr. A. J. Walker that the proposition be circularized among the members for their comments. Both the motion and amendment were accepted.

The business meeting adjourned at 9:15 a.m.

MEETING OF THE BOARD OF DIRECTORS NMS

Sunday, November 6, 1949 Hotel Peabody Memphis, Tennessee

The Board of Directors met in Room 212, Hotel Peabody, at 3:00 p.m. Members present were Dr. Wendell Gingrich, President; Dr. Paul F. Russel, President-Elect; Dr. E. Carroll Faust, Vice President; Mr. W. H. W. Komp, Mr. H. W. Van Hovenberg, and Dr. E. Harold Hinman, Directors; and Dr. Martin D. Young, Secretary. Dr. Justin M. Andrews was present by invitation.

President Gingrich appointed committees to convass the ballots for election of officers, to audit the books of the Society, and to draw appropriate resolutions.

The actions of the Board of Directors during 1949 were as follows: approved committees appointed by the President; agreed to meet with the American Society of Tropical Medicine and the American Academy of Tropical Medicine in Memphis, 1949; voted to affiliate with the National Society for Medical Research without financial contribution; approved the suggestions of the Committee on Lectureship and Award; and approved the slate of candidates proposed by the Committee on Nominations.

The report of the Secretary-Treasurer was accepted. Dr. Young was reappointed to serve as Secretary-Treasurer for one year.

The report of the Committee on Policy was accepted.

The recommendations of the Committee on Lectureship and Award were approved: that an award be given every 3 years and that it be called the Joseph Augustin LePrince Award. The incoming President was asked to select a committee to suggest ways of financing the award.

The Board of Directors instructed the Secretary to write to the American Foundation for Tropical Medicine expressing the Society's thanks for past help and for possible future assistance.

The Directors approved honoraria in the amount of $200 for the Secretary's stenographer and $25 for the Editor's stenographer. Funds up to $1200 were authorized for stenographic help in the Secretary's office during 1950, to be disbursed only if necessary.

It was recommended that the Secretary continue his $5,000 bond during 1950. Approval was granted for the Secretary to handle all routine business of the Society.

Upon the request of the Editorial Board, the Directors authorized the printing of 96 pages per issue in the 1950 Journal.

The Board recommended that the National Malaria Society indorse the nomination of General Gorgas to the New York University Hall of Fame, and that members in each state contact their electors. The Secretary was appointed to get the names of electors.

The Directors favored meeting in Savannah in 1950 if this was agreeable to the American Society of Tropical Medicine and the American Academy of Tropical Medicine. The Secretary was instructed to act on behalf of the National Malaria Society. Doctor Russell suggested a 4-day meeting with one day for a demonstration trip to the U. S. Public Health Service Laboratory, Savannah, Georgia.

For 1951, it was felt that the possibility of meeting in Havana should be explored.

Doctor Andrews proposed that the Society set forth criteria which could be used by official agencies to determine the time when malaria ceases to be an endemic disease in the United States. After discussion, it was moved that the Board recommend that the National Malaria Society consider the development of criteria for determining the time when malaria ceases to be an endemic disease and that the incoming President appoint a committee to draft these criteria.

The Board of Directors approved 5 applications for membership.

The meeting adjourned *sine die* at 5:30 p.m.

NEW MEMBERS OF THE NATIONAL MALARIA SOCIETY

1949

(Additions to the Lists Published in the Journal:
4(4): 351–64—December, 1945
7(1): 79–83—March, 1948
8(1): 112–114—March, 1949)

BLANKS, Mr. Charles Preston, Jr., Institute Inter American Affairs, c/o U. S. Embassy, Quito, Pichincha, Ecuador

BRIDGES, Mr. C. Bradley, Box 372, Cordele, Georgia

CAMBOURNAC, Dr. Francisco José, Avenida Dr. Cambournac No. 4, Sintra, Portugal

CHAFFEE, Major Elmer F., 714 Gist Avenue, Silver Spring, Maryland

COX, Mr. Dennis L., Jr., Sandy Bluff Road, Mullins, South Carolina

CRUZ, FERREIRA, Dr. Fernando Simões, Instituto de Medicina Tropical, Lisbôa, Portugal

DUQUE, Dr. Merced S., 10-A Santiago, Paco, Manila, Philippines

EJERCITO Y LIZA, Dr. Antonio, Malaria Section, Department of Health, Manila, Philippines

FIFE, Mr. Earl H., Jr., Dept. of Serology, Army Medical Department Research & Graduate School, Army Medical Center, Washington 12, D. C.

FOGG, Mrs. Virginia H., 244 S. Cleveland, Apt. # 24, Memphis, Tennessee

FONSECA, Mr. James R. C., 34–51 82nd Street, Jackson Heights, L.I., N.Y.

JEFFERY, Dr. Geoffrey M., P. O. Box 356, Milledgeville, Georgia

GOFF, Mr. George Allen, Route 8, Box 250, San Jose Blvd, South Jacksonville, Florida

GONZALES-MUGABURU, Dr. Luis, Arda. Arequipa 315, Lima, Peru

GRANT, Mrs. Jean S., 871 Beaverbrook Drive, N. W., Atlanta, Georgia

HYDE, Dr. Henry van Zile, 107 Battery Lane, Bethesda, Maryland

JENNEY, Dr. Elliott Ross, 12 Beatricia Road, Orinda, California

JETTMAR, Dr. Heinrich Manfred, Graz, (Styria) Austria, Hygienisches Institut, Universitatsplatz 4, Graz, Austria

JORDAN, Miss Helen Berry, Department of Zoology, University of California, Berkeley 4, California

HUNNINEN, Dr. Arne V., Mount Union College, Alliance, Ohio

KRISHNA, Dr. Anwikar Anant, Dhantoli, Nagpur, Central Provinces & Berar, India

LIVADAS, Dr. A. Gregory, 37, Limnou Street, Athens, Greece

MISSIROLI, Dr. Alberto, Via Carlo Fea 15, Rome, Italy

MACDOUGALL, Dr. Mary Stuart, Agnes Scott College, Decatur, Georgia

MACKERRAS, Dr. Ian Murray, Queensland Institute of Medical Research, Herston Road, Valley, Brisbane, Queensland, Australia

MEHRA, Dr. A. Torab, Avenue Shahreza, Opposite Estakhr Ramsor, Tehran, Iran

MONAGHAN, Mr. Peter J., 2476 W. Highland Avenue, Milwaukee 3, Wisconsin

MRACEK, Mr. James Frank, 1036 Finkbine Park, Iowa City, Iowa
NAIR, Major C. P., Malaria Institute of India, 22, Alipore Road, Delhi, India
NEWMAN, Miss Doris, 1237 33rd Street, N. W., Washington 7, D. C.
NORTON, Dr. J. W. R., State Health Officer, State Board of Health, Raleigh, N. C.
RAFFAELE, Dr. Giulio, Instituto di Malariologia, Policlinico, Roma, Italy
RAMAKRISHNAN, Dr. S. P., Malaria Institute of India, South India Branch, Conoor, India
RAY, Dr. A. P., c/o Dr. Fred L. Soper, 2001 Connecticut Avenue, Washington 8, D. C.
REHN, Dr. John W. H., School of Tropical Medicine, San Juan 22, Puerto Rico
ROBINSON, FRANCES O., Christ Hospital Institute of Medical Research, Cincinnati Ohio
SAFFAR, Dr. A. Samim, 2/2/1 Alwadhia, Baghdad, Iraq
SCHAEFFER, Dr. Kaethe, 410 Riverside Drive, New York 25, N. Y., c/o Kaskell,
SHORTT, Dr. Henry Edward, London School of Hygiene & Tropical Medicine, Keppel St., London W. C. 1, England
THARP, Miss Annekay, 1724 Nelson Avenue, Memphis 4, Tennessee
THOMSON, Mr. Junius Richard, 632 E. Lake Drive, Decatur, Georgia
*TRAVIS, Dr. B. V., Dept. of Entomology, Cornell University, Ithaca, New York
VAUCEL, Dr. Marcel Augustin, Ministere de la France d'Outre-Mer, Paris, France
VISWANATHAN, Dr. Dharmavadani Krishnier, Assistant Director of Public Health (Malaria), Poona, India
WEEN, Miss Goldye, 543 West 24th Street, Jacksonville, Florida
WONG, Mr. Chester, 312 N. Boyle Avenue, Los Angeles 33, California

* Membership effective 1950.

BOOK REVIEW

MALARIOLOGY—Comprehensive Survey of All Aspects of this Group of Diseases from a Global Standpoint: By Sixty-five Contributors. Edited by Mark F. Boyd. Volumes I and II. pp. 1 thru 1,643; figs. 1 thru 436. Philadelphia and London: W. B. Saunders Company, 1949. Price $35.00 set.

Colonel Sir Rickard Christophers in the foreword to these two volumes says "What one author, however, might find it difficult or impossible to achieve, a number of authorities each in his own field might accomplish. It is on such a basis that the Editor has planned the present volume and for this, as will be seen from the list of contributors, he has secured the cooperation of many recognized authorities throughout the world who have recently actively engaged on the particular aspect of malaria with which they deal, thus making available not only the most up-to-date information in practically every branch of malariology but also a wealth of factual experience that could probably never have been attained in any other way."

The material in these two volumes is divided into five sections and 70 chapters with two appendices and an author and a subject index. Section I, 21 pages, is devoted to a historical review: Section II, 196 pages, on parasitology is divided in 8 chapters: Section III, 326 pages, on the definitive hosts has 13 chapters: Section IV, 610 pages, on the intermediate host has 25 chapters and Section V, 317 pages, on control and eradication has 21 chapters. References are listed at the end of the chapter in which they occur.

The books are good examples of what the American book publisher can do. The text has been set in 10 pt. type and arranged two columns to the page. Excellent reproduction and a good quality paper make the illustrations clean cut and clear. The typography and the arrangement of material are excellent throughout. The two volumes are bound in brown cloth.

These two volumes are books that every malariologist should have and will want. Although $35.00 seems high it is just about 2 cents a page and compares favorably with the price of other technical books.

Dr. Boyd and the publishers are to be congratulated for making available these two excellent volumes.

Introduction to Parasitology. By ASA C. CHANDLER. Eighth edition. Pp. xii plus 756. Figs. 273. John Wiley and Sons, Inc., New York. 1949. $6.00

Parasitologists will welcome this new edition of a very popular textbook on parasitology. The scope of the book has been enlarged somewhat over that of the last edition. The additional material will prove useful to those who are interested in the parasites of non-human hosts and their possible relationships to those in man. Particularly gratifying is the inclusion of recent information, such as the newest chemotherapeutic agents in malaria, some of which became available in the same year the book was published.

The material in the book comprises 28 chapters as follows: two chapters covering the introduction and a general discussion of parasites; eight chapters on protozoa, one chapter of which is on spirochetes and part of another chapter concerns arthropod-borne organisms other than protozoa, such as rickettsias, viruses, and bacteria; nine chapters are on helminths; and, finally, nine chapters are concerned with arthropods.

The classification and taxonomy of the various groups of parasites are given, with simple keys especially on the arthropods. For the most important parasites, life cycles, epidemiological factors, host-parasite relationships, and principles of treatment and control are considered. Particular emphasis has been placed on the fundamental biological principles rather than on specialized details.

The book is written in the same entertaining style as the former editions. This, coupled with the accurateness of the factual information, marks it as one of the most readable books dealing with a specialized subject. Not only is it admirably suited as an introductory text in parasitology courses, but parasitologists will turn to it to find prosaic facts presented in an interesting and stimulating manner.

Malariologists will find the human plasmodia dealt with in one chapter of 35 pages and the mosquito vectors covered in another chapter of 46 pages. This information is of necessity generalized but is well-selected and brings the reader up-to-date in a field that is rapidly expanding.

The references listed at the end of the chapters do not include all of those mentioned by name and date in the text. Even though the author clearly sets forth his reasoning for this in the preface, one still regrets not being able to refer to the complete reference to pursue a point that has been so intriguingly presented in the text.

Although the book is amply illustrated with line drawings which supplement the text descriptions, the average quality of these seem to be somewhat poorer than one would expect, and some few, such as the figure comparing the three species of malaria parasites, are inferior.

The reviewer found a paucity of misstatement of facts. It was noticed, however, that in presenting a diagrammatic sketch of the life cycle of *Plasmodium vivax*, posterythrocytic stages of the exoerythrocytic cycle were shown; these must be hypothetical, although it is not so stated in the figure legend or in the text.

The book is printed on good paper and is attractively bound. It will continue to be a standard in the field of parasitology, both for those who are being introduced to a new and, too often, a most complicated subject, and for those specializing in a particular phase of parasitology who wish to read of kindred parasites in related fields.—Martin D. Young.

Schedule of Laboratory Training Courses

May 8–May 12	LABORATORY DIAGNOSIS OF RABIES
May 22–May 26	LABORATORY DIAGNOSIS OF BACTERIAL DISEASES (DIRECTORS)
May 29–June 2	LABORATORY DIAGNOSIS OF MYCOTIC DISEASES (DIRECTORS)
June 5–June 9	LABORATORY DIAGNOSIS OF TUBERCULOSIS (DIRECTORS)
June 12–June 16	LABORATORY DIAGNOSIS OF PARASITIC DISEASES (DIRECTORS)
July 24–Aug. 4	LABORATORY DIAGNOSIS OF MYCOTIC DISEASES PART 1. CUTANEOUS AND SUBCUTANEOUS FUNGI
Aug. 7–Aug. 17	LABORATORY DIAGNOSIS OF MYCOTIC DISEASES PART 2. SYSTEMIC FUNGI
Aug. 21–Sept. 7	LABORATORY DIAGNOSIS OF TUBERCULOSIS
Sept. 11–Sept. 22	LABORATORY DIAGNOSIS OF BACTERIAL DISEASES GENERAL BACTERIOLOGY, PART 1.
Sept. 25–Oct. 6	LABORATORY DIAGNOSIS OF BACTERIAL DISEASES GENERAL BACTERIOLOGY, PART 2.
Oct. 9–Oct. 13	LABORATORY DIAGNOSIS OF ENTERIC DISEASES PART 1. INTRODUCTORY ENTERIC BACTERIOLOGY
Oct. 16–Oct. 27	LABORATORY DIAGNOSIS OF ENTERIC DISEASES PART 2. ADVANCED ENTERIC BACTERIOLOGY
Sept. 18–Oct. 6	LABORATORY DIAGNOSIS OF PARASITIC DISEASES PART 1. INTESTINAL PARASITES
Oct. 9–Oct. 27	LABORATORY DIAGNOSIS OF PARASITIC DISEASES PART 2. BLOOD PARASITES
Nov. 6–Nov. 10	SEROLOGICAL DIAGNOSIS OF RICKETTSIAL DISEASES
Nov. 13–Nov. 24	IDENTIFICATION OF MEDICALLY IMPORTANT ARTHROPODS
Nov. 13–Nov. 17	VIRUS ISOLATION AND IDENTIFICATION TECHNIQUES
Nov. 20–Nov. 24	LABORATORY DIAGNOSIS OF INFLUENZA
Nov. 27–Dec. 1	LABORATORY DIAGNOSIS OF RABIES
Dec. 4–Dec. 15	LABORATORY DIAGNOSIS OF TUBERCULOSIS
By Special Arrangement	LABORATORY DIAGNOSIS OF MALARIA LABORATORY DIAGNOSIS OF VIRUS DISEASES PHAGE TYPING OF SALMONELLA TYPHOSA

Information and applications should be requested from the Chief, Laboratory Division, Communicable Disease Center, 291 Peachtree St., N.E., Atlanta, Ga.